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Behavior and the Temporal Rhinencephalon in the Monkey.
A. Earl Walker, Alfredo F. Thomson and J. Donald McQueen 65

Filter Paper Electrophoresis of Human Hemoglobins With Special Reference to the Incidence and Clinical Significance of Hemoglobin C. Ernest W. Smith and C. Lockard Conley 94

Studies of Extrahepatic Biliary Obstruction in the White Mouse by Fluorescence Microscopy.
Allan L. Grafflin and Verne E. Chaney, Jr. 107

Book Reviews 141

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BEHAVIOR AND THE TEMPORAL RHINENCEPHALON IN THE MONKEY[1]

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Received for publication May 13, 1953

INTRODUCTION

Before the turn of the century, Brown and Shäfer (2) described striking behavioral changes in monkeys as the result of bilateral temporal lobectomy. Their observations stimulated great local interest at that time but were soon forgotten. Half a century later, Klüver and Bucy (7), having rediscovered these behavioral alterations, came upon the report of the English investigators in their search of the literature. This time, the observation came at a time when neurologists were particularly interested in correlating behavior and brain, and so it stimulated both basic and clinical research into the functions of the temporal cortex. Klüver and Bucy described the syndrome produced by temporal lobectomy as 1) a psychic blindness or inability to recognize and detect the meaning of objects on the basis of visual criterion alone, 2) strong oral tendencies manifested by carrying all objects to the mouth or nose, 3) a tendency to react to every visual stimulus as if an irresistible impulse were present to contact all objects in sight (hypermetamorphosis), 4) loss of emotional response to situations normally inducing fear or anger, 5) an increase in the amount and diversity of sexual behavior, and 6) a change in dietary habits so that the animal became carnivorous.

These striking behavioral disturbances followed bilateral temporal lobectomy but were not seen when the ablations were limited to the lateral temporal gyri. In fact Klüver recently states (5, p. 163) "Although these experiments have not led to definite conclusions, they strongly suggest that the hippocampal formation and its connections play an important role in the production of the symptoms."

This investigation was undertaken to determine the role of the cortical structures on the inferior and medial aspect of the temporal lobe in the production of this syndrome. A preliminary report of the clinical observations was made three years ago (12), at which time some animals were still alive; this paper presents the detailed clinical findings and anatomical controls of the entire series.

[1] The histological preparation of these brains was aided by a grant (B68) from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service.

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METHODS

Eleven macaque monkeys (macaca mulatta), presumably 2 years of age, were used in this study. The observations of behavior were noted under a variety of conditions in order to test the reaction of the animal to different forms of stress.

1. In a large cage (1 x 2 x 3 meters)
   (a) the monkey's general motor abilities—running, climbing, jumping.
   (b) The animal's reaction to the presence of other monkeys and other animals such as cats, guinea pigs, mice and man.
   (c) The animal's sexual behavior.
   (d) The animal's reaction to edible and non-edible objects.
2. In a small cage
   (a) The reaction of the isolated animal to food.
   (b) Its reaction to the observer.
3. In a chair, the monkey sitting upright and restrained by straps about the lower abdomen and upper chest.
   (a) The reflex motor status.
   (b) Visual fields.
   (c) Other neurological examinations.
4. On an animal board, the arms and legs restrained and a collar preventing undue movement of the head.
   (a) The reaction of the monkey to changes in its environment.
   (b) To various sensory stimuli—pain, auditory and olfactory.

Electroencephalograms were made at intervals on an 8 channel Grass electroencephalograph.

The weight of the animal, determined monthly, served as a rough guide to its general physical condition.

Control observations as mentioned above were made daily or every other day, by one of us, (A. F. T.) for periods from 1 week to 2 months prior to any operative procedure, and after each operative procedure for at least two months unless otherwise stated. During the later convalescence, the animals were examined in the above routine manner every two weeks for the first six months and then approximately once a month. Because interest was centered upon behavior, the "personality" of each monkey was carefully assessed before operation. Each animal, like each human being, has certain personal characteristics. In general, the monkey presents a pseudo-aggressive wild reaction to the presence of man—snarling face, bared teeth, retracted scalp and alerted ears, protruding head, feigning aggressive movements of the legs and body, signs of sympathetic discharge—dilated pupils, accelerated breathing and defecation—but these responses are seen in varying degrees in different animals. Moreover, the social conduct of the monkey with its kind places upon it a distinct personality. A knowledge of the normal characteristics of a monkey was a prerequisite to the recognition of its abnormal behavior. In the protocols to save space only an abstract of the positive findings of the routine testing which was carried out will be given.

The operations were performed under general anesthesia induced by pentobarbital sodium given intraperitoneally. They were carried out in two stages at an interval of approximately six days, with the exception of monkey T9 which had a second operation 20 days after the first. In every case the first operation was performed on the left side. The incision, perpendicular to the zygomatic arch, was made half way between the external canthus of the orbit and the anterior margin of the external ear. The zygoma was partially extirpated; a trephine opening was made on the inferior part of the squamous portion of the temporal bone and was enlarged with the rongeur. The inferior portion of the temporal lobe was exposed by an

incision of the dura mater. By means of aspiration various portions of the inferior and medial part of the temporal lobe were removed. There were no postoperative complications such as infection or hemorrhage in any of the animals (Fig. 1).

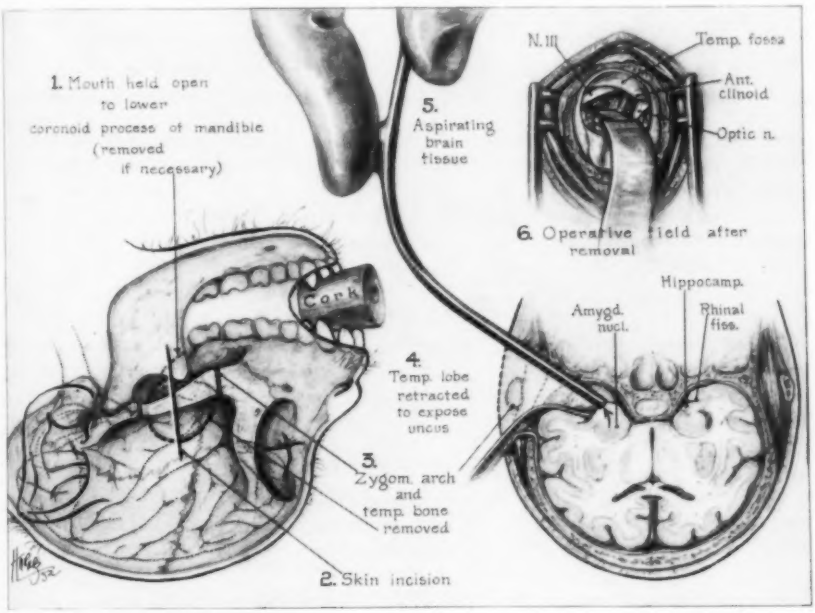

FIG. 1. The surgical approach for excision of temporal rhinencephalic structures with minimal damage to neocortex.

The abbreviations used in the illustrations are as follows:

A — nucleus amygdala
AC — commissura anterior
C — claustrum
G — nucleus geniculatus lateralis
H — hippocampus
IT — sulcus temporalis inferior
L — corpus Luysii
NC — nucleus lenticularis
OC — chiasma optica
OI — sulcus occipitalis inferior
OL — sulcus orbitalis lateralis
OM — sulcus orbitalis medialis
OT — sulcus occipito-temporalis
P — pons
R — sulcus rhinalis
S — sulcus Sylvii
ST — sulcus temporalis superior

T — tractus olfactorius
TH — thalamus
TO — tractus opticus

All brains were examined grossly, photographed and cut in serial sections at 20 μ and every 25th section stained for cells by Nissl's technique and an adjacent section for myelin, by the Smith-Quigley technique. Where indicated, additional sections were stained and examined.

PROTOCOLS

Experiment 1. (M. T79) A somewhat aggressive, female macaque; medial temporal lesions damaging superficially the anterior basal half of the amygdaloid complex; no alteration in behavior after operation.

This female macaque came under observation on March 17, 1949 (weight 2.5 kg.). The animal had no abnormalities on neurological examination and reacted well to the various forms of stimuli.

Operations

The animal had a left temporal craniotomy on April 28, 1949 with removal of a small area of temporal cortex just lateral to the uncus. There was no behavioral change following this procedure. On May 6, 1949 a similar procedure was carried out on the right side. The lesions were verified by autopsy and histological study.

Pathological Anatomy

Right hemisphere. The lesion of the right temporal lobe lies along the superior temporal fissure measuring 1.0 cm. laterally and 0.4 cm. anteroposteriorly. It extends 3 mm. mediallv to the rhinal fissure injuring an area (3 x 2 mm.) of the uncus. The base of the lesion is depressed 2 mm. below the surface of the convolutions.

An examination of serial sections of the brain confirms the gross location of the lesion. It damages slightly the cortical amygdaloid transitional area, the basal amygdaloid nucleus and the parts lying between it and the rhinal sulcus. The cortex between rhinal and hippocampal fissures is damaged.

Left hemisphere. On the left side the lesion has a roughly circular outline about the end of the superior temporal sulcus. It extends to the margin of the uncus which is superficially damaged. The crater of the lesion is slightly depressed below the level of the temporal cortex.

An examination of serial sections of the brain confirms the gross location of the lesion. It has superficially damaged the cortical amygdaloid nucleus, and in one plane there is a linear incision across the basal amygdaloid nucleus just rostral to the magnocellular part of the nucleus. The posterior half of the amygdaloid complex is intact. The cortex between the rhinal and hippocampal fissures is destroyed (Fig. 2).

Postoperative State

May 9, 1949. The animal is aggressive and cannot be caught without gloves. From the farthest corner of the cage, it follows suspiciously every movement of the observer. With other monkeys it is belligerent. No sexual activity is noticed.

June 21, 1949. The animal is quite wild, snarling and biting when molested. In the large cage, it watches from the farthest corner, baring its teeth if approached. When tested with a variety of objects, the edible ones are snatched and eaten, and the others ignored.

Until July 7, 1949, the animal was observed daily, but it remained wild and aggressive. No alterations were noted in its sexual patterns, or in its reactions to other animals. After that date it was studied monthly until it died on November 6, 1949 (six months after the

second operation) presumably of a gastro-intestinal infection. There was no change in its behavior during this period.

Experiment 2. (M. T73) A timid female macaque; bilateral ablations of the medial temporal lobes; superficial damage to the left cortical amygdaloid transitional area only; anterior hippocampus destroyed bilaterally; after operation no behavioral changes.

This animal, a female, came under observation March 8, 1949 (weight 2.5 kg.). The animal had no abnormalities on neurological examination and responded well to all sensory stimuli.

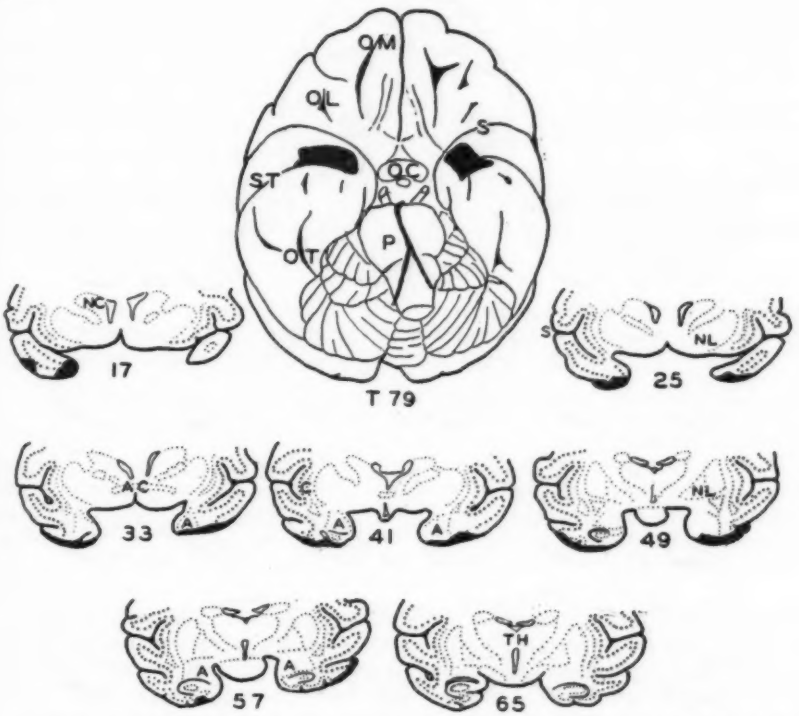

FIG. 2. Experiment 1. Sketches of the cerebral hemispheres and serial sections of the lesion to show the extent of neocortical and rhinencephalic involvement.

Operations

A left temporal craniotomy was performed on April 29, 1949 and cortex on the medial aspect of the temporal lobe was ablated. The animal's behavior and neurological state were unchanged. A similar procedure was carried out on the right temporal lobe May 7, 1949.

Pathological Anatomy

Right hemisphere. On the right side the lesion lies in the second temporal convolution. It measures 1.3 cm. laterally and 1.2 cm. anteroposteriorly. The lesion lies between the rhinal and collateral fissures and the superior temporal fissure.

Examination of serial sections confirms the gross location of the lesion. In no place did it involve the amygdaloid complex; only the cortex of the hippocampus lying between the rhinal and hippocampal fissures is damaged. The inferior temporal gyrus is the site of the main but superficial lesion.

Left hemisphere. On the inferior surface of the left temporal lobe is a rectangular ablation extending from the hippocampal sulcus laterally for a distance of 1 cm. Along the medial margin it measures 9 mm. anteroposteriorly and at its lateral margin 5 mm. The base of the ablation is depressed for 2 mm. below the normal contour of the brain. Most of the cortex

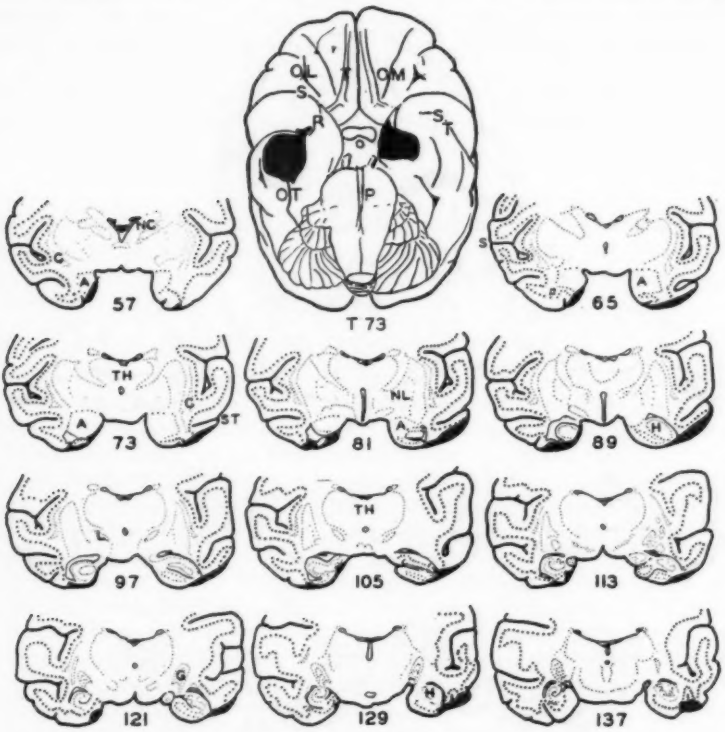

FIG. 3. Experiment 2. Sketches to show the temporal lobe lesions.

medial to the rhinal sulcus has been removed, except for a triangle anterior to the optic chiasm. The tip of the temporal lobe is undamaged.

The serial sections of the brain confirm the location of the lesion which superficially damages the cortical amygdaloid transitional area but no other elements of the amygdaloid complex. The anterior part of the hippocampal cortex lying between the hippocampal and rhinal fissures is ablated but the hippocampal formation otherwise is intact. The temporal horn of the ventricle is dilated (Fig. 3).

Postoperative Status

May 15, 1949. The animal's behavior is the same as before its operation. It responds normally to sensory stimuli. Its sexual activity is normal.

June 21, 1949. The monkey has no difficulty in differentiating edible from inedible objects. The animal was observed at frequent intervals until the time of sacrifice, November 4, 1949. No behavioral changes were noted in this monkey at any time.

Experiment 3. (M. T85) An aggressive male macaque monkey; extensive medial temporal lobe ablations including anterior half of right amygdala and ventral half left amygdala and rostral hippocampus; after operation temporarily nonchalant but apprehensive; later aggressive.

This male macaque came under observation March 8, 1949, at which time it weighed 2.4 kg. The animal had no abnormalities on neurological examination.

Preoperative State

Reaction to man. The monkey displayed typical pseudo-aggression, snarling but apprehensive when the observer was present.

Reaction to other animals. This animal was aggressive to both male and female monkeys. The monkey was observed to have frequent sexual relations with any one of several females in the colony, but it never exhibited interest in the males.

When a cat was put in its cage, the monkey attempted to handle it until the cat, becoming angry, clawed; thereupon the monkey lost interest in its feline cagemate.

Reaction to food. The monkey chose edible vegetables readily ignoring all inedible objects.

Operations

After almost two months' observation, this monkey had a left temporal lobe ablation on May 2, 1949, and a right temporal lobe ablation on May 9, 1949. The animal had an uneventful convalescence after each operation.

Pathological Anatomy

Right hemisphere. The right temporal lesion measures 1.3 cm. in width and 0.8 cm. in length. It extends from the temporal tip where it reaches to within 3 mm. of the Sylvian fissure to the occipito-temporal fissure. Medially it begins 4 mm. from the hippocampal fissure, the medial portions of the uncus and hippocampal gyri being preserved. The lesion extends laterally from the superior temporal gyrus across the superior and inferior temporal sulci to the occipito-temporal sulcus. The crater is 3 mm. below the normal contour of the temporal cortex.

Upon examination of the serial sections the gross location of the lesion is confirmed. The cortical amygdaloid complex is largely ablated. An incision passes from the lateral aspect of the amygdaloid complex to the incisura temporalis leaving an island of undamaged magnocellular basal accessory nucleus. Further posteriorly the basal accessory nucleus is little damaged and the lateral amygdaloid nucleus is slightly damaged. The accessory basal nucleus is damaged only on its ventral aspect. The cortex between the rhinal and hippocampal sulci is ablated but there is only superficial damage to the hippocampal complex.

Left hemisphere. The lesion of the left temporal lobe is larger than that on the right side, measuring 1.4 x 0.9 cm. The ablation extends from within 3 mm. of the Sylvian fissure in the superior temporal gyrus caudally to the occipito-temporal gyrus. On the medial aspect of the lobe the uncus is almost completely removed as well as the cortex on both walls of the rhinal fissure except that on its tip. Laterally the lesion extends to the inferior temporal convolution, and for some distance lies along the inferior temporal sulcus. The crater is only 2 to 3 mm. below the contour of the temporal cortex.

In serial sections of the cortex, the gross location of the lesion is confirmed. There is no deep laceration or softening. The anterior and dorsal portion of the amygdaloid nuclear complex is preserved, but at about its middle third, the ventral half of the amygdala has

been ablated leaving intact only the dorsal half of the magnocellular part of the basal nucleus and the adjacent lateral and accessory basal nuclei, which appear to have undergone retrograde cell degeneration. The hippocampus and overlying cortex have been ablated to the level of the corpus Luysii. In its caudal third the lateral nucleus is also involved in its inferior moiety. This ablation continues to the posterior extremity of the complex and into the hippocampus, the anterior half of which is almost entirely removed (Fig. 4).

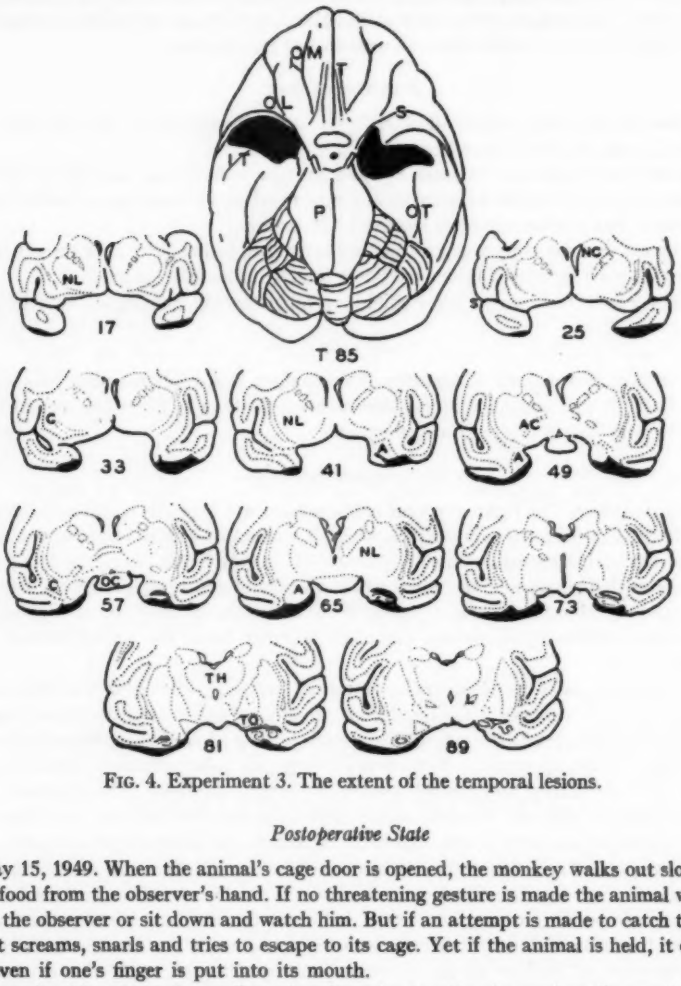

FIG. 4. Experiment 3. The extent of the temporal lesions.

Postoperative State

May 15, 1949. When the animal's cage door is opened, the monkey walks out slowly and takes food from the observer's hand. If no threatening gesture is made the animal will walk about the observer or sit down and watch him. But if an attempt is made to catch the monkey, it screams, snarls and tries to escape to its cage. Yet if the animal is held, it does not bite even if one's finger is put into its mouth.

The animal is attracted sexually to females only; in spite of a good penile erection, copulation appears to be perfunctory.

The animal has no apparent abnormalities on neurological examination.

May 17, 1949. Before the animal is fed the following items on a wooden tray are presented:

gauze, match, string, apple, bakelite knob, cardboard square, apple cotton ball, circle cardboard, nut, potato and a piece of feces. With its right hand the monkey picks up the food without hesitation. After a few minutes it picks up the bakelite knob, smells it and throws it away. No attention is paid to the other items.

When a piece of apple, covered with 2 pieces of cardboard is presented, the monkey throws the cardbard away, without carrying it orally, then with its right hand picks up and eats the apple. The whole operation requires about 4 seconds.

For the next two weeks the animal was examined daily. No further alterations were detected in its condition. Its weight on July 7, 1949 was 2.4 kg. Three electroencephalograms made during this period were all reported normal.

August 12, 1949. The animal's neurological status appears normal. It is still tame enough to pick food from the observer's hand and eat it perched nearby, but is more aggressive and apprehensive than formerly. The animal has been observed in frequent sexual relations with females but probably not more often than normal.

September 16, 1949. The animal is quite lively, jumping out of its cage and running to the far corner of the room. It is quite aggressive and cannot be caught or held with one's bare hand. It displays no unusual sexual activity. Upon repeating the food test, the animal rapidly grabs the edible articles and ignores the others.

October 19, 1949. The animal is now almost as aggressively wild as before its operations. In fact one notices little abnormal in its behavior.

The general condition of the animal remained unchanged until January 2, 1950, eight months after the second operation when it was sacrificed.

Experiment 4. (M. T84) A wild aggressive male monkey; bilateral lesions of medial temporal lobe involving the ventral two thirds of the amygdaloid complex; after operation apathetic and tame; tameness gradually decreased but apathy persisted for 18 months.

This male macaque came under observation March 9, 1949 (weight 2.3 kg.). The animal had no abnormalities on neurological examination.

Preoperative Status

Reaction to man. The animal was quite wild and aggressive whenever the observer approached his cage. When restrained on the examining board it would violently shake the board from time to time.

Reaction to other animals. The animal was aggressive with other monkeys and frequently paid attention to them, picking fleas from their bodies. At times it would present to other animals but did not have homosexual relationships. It had frequent sexual relationships with any of the females. When a cat was introduced in its cage the animal attempted to touch it but after the cat clawed, the monkey became indifferent to its presence.

Reaction to food. The animal quickly grabbed edible objects presented but refused all inedible objects.

Operations

On May 4, 1949, a left temporal craniotomy was performed and the medial temporal cortex ablated. The animal appeared normal after this procedure. The same type of operation was carried out on the right side on May 9, 1949.

Pathological Anatomy

Right hemisphere. On the right side is a roughly quadrilateral lesion, 1.2 cm. in width and 1.0 cm. anteroposteriorly, on the medial surface of the temporal lobe. It extends from the hippocampal fissure laterally to the superior, medial and inferior temporal gyri. Anteriorly

the lesion reaches almost to the Sylvian fissure and posteriorly to the level of the rostral margin of the pons.

Examination of serial sections of the brain confirms the gross location of the lesion. On the right side the cortical nucleus, the basal accessory and lateral nuclei of the amygdala are severely damaged, only the dorsal portion of the complex remaining. The anterior part of the hippocampal gyrus and hippocampus is also damaged.

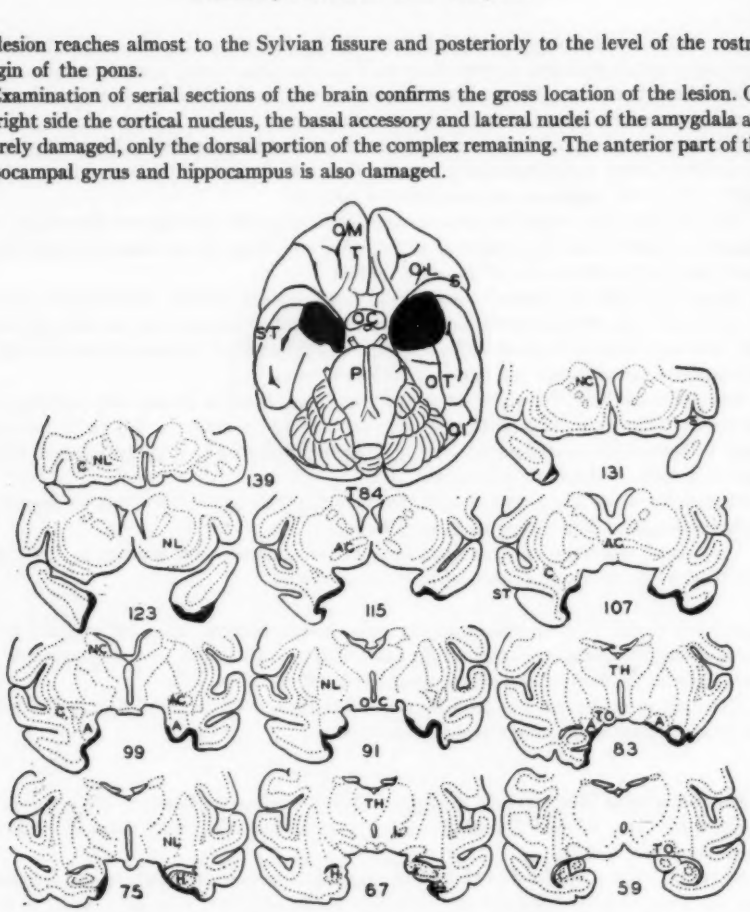

FIG. 5. Experiment 4. Sketches to show the lesions and their extent.

Left hemisphere. On the left side, the lesion approximately 1.8 cm. in diameter extends from the level of the optic chiasm along the superior temporal gyrus, passes caudally to the inferior temporal fissure and then medially in the plane of the anterior part of the pons to the hippocampal fissure. The lesion has removed all the cortex within the above area.

Examination of serial sections of the brain confirms the extent of the lesion. The cortical nucleus and the inferior part of the main amygdaloid complex has been removed leaving intact only the dorsal third. The hippocampal gyrus between the rhinal and hippocampal sulci has been ablated including the anterior part of the hippocampus (Fig. 5).

Postoperative State

May 15, 1949. The animal is much quieter than before its operation, sitting for long periods of time in one place in its cage. It pays little attention to other monkeys, and has little sexual activity. When a guinea pig or cat is put in its cage, it is indifferent to their presence.

Yet it responds normally to auditory and olactory stimuli, and when its tail is pinched, it snarls and jerks the tail away. The animal will allow itself to be caught without an attempt at flight. Even if the observer places his finger on the animal's lips, it is not bitten.

During the next three months the animal remained in much the same apathetic and "tame" state, although its sexual activity gradually increased to normal by July 7, 1949.

August 8, 1949. The animal remains quite tame and makes no effort to avoid the examiner in the cage. When caught, it lies quietly, making a crying noise rather than a growl or snarl, and never bares its teeth. It shows normal sexual interest, but is not normally reactive to other animals—i.e. does not search their fur for fleas, etc. In general its movements are slow and deliberate, a custom perhaps exaggerated by frequent yawning.

When presented with a series of objects, it picks and eats the pieces of apple with its right hand slowly. Then the cotton is carried to its nose and discarded. The potato is then eaten with the left hand. Having consumed the edible objects the animal pays no attention to the other objects but tries to steal food from the adjacent cage. When the covered apple was presented, the animal deliberately removed the cardboard and within 25 seconds had eaten the apple.

August 23, 1949. The animal is still rather quiet and prefers to sit silently and motionless in the front of its cage.

During the next six months there was little change in the behavior of the animal. At the end of this time it could still be caught with one's bare hands without danger of being bitten. It was not quite as tame as in August but still showed no signs of affection for other monkeys, although its sexual activity appeared to be normal.

The animal was sacrificed November 13, 1950, eighteen months after its second temporal operation. At that time the monkey, in excellent physical condition, still was tamer than the usual macaque but not sufficiently subdued to be caught without gloves. It had been somewhat less active than the normal monkey but its sexual activity seemed normal.

Experiment 5. (M. T83) An aggressive female macaque; ablation of medial surface temporal lobes including a part of the right cortico-amygdaloid transition area and posterior part of the amygdaloid complex and on the left side of the rostral and middle third of the complex; after operation tame and playful for 4 months, then more aggressive.

This female macaque monkey came under observation March 10, 1949 (weight 2.6 kg.). The animal had no abnormalities on neurological examination.

Preoperative State

Reaction to man. The animal was quite aggressive and wild; when restrained on an examining board the animal would shake it violently.

Reaction to other animals. The animal appeared to be quite aggressive with other monkeys; it had heterosexual intercourse frequently.

Reaction to food. The animal usually took edible objects with its right hand and ignored completely inedible material.

Operations

On May 5, 1949, a left temporal craniotomy was performed and an area of cortex on the medial surface of the temporal lobe was ablated. The animal had no change in behavior. On May 10, 1949 a similar right medial temporal ablation was made.

Pathological Anatomy

Right hemisphere. In the right temporal lobe is a narrow (0.4 cm.) and shallow cortical excision extending medially from the inferior temporal sulcus across the rhinal fissure to the hippocampal fissure, a distance of 1 cm.

Examination of the serial coronal sections confirms the extent of the lesion which begins anteriorly as a superficial lesion just medial to the rhinal fissure damaging the cortico-amygdaloid transition area. Slightly posteriorly the lesion extends laterally to damage the cortex between the rhinal and hippocampal fissures and involves the base of the amygdaloid complex and the hippocampal structures.

Left hemisphere. The almost circular lesion extends approximately 0.5 cm. in diameter medially and posteriorly from the tip of the superior temporal sulcus. A tail-like extension

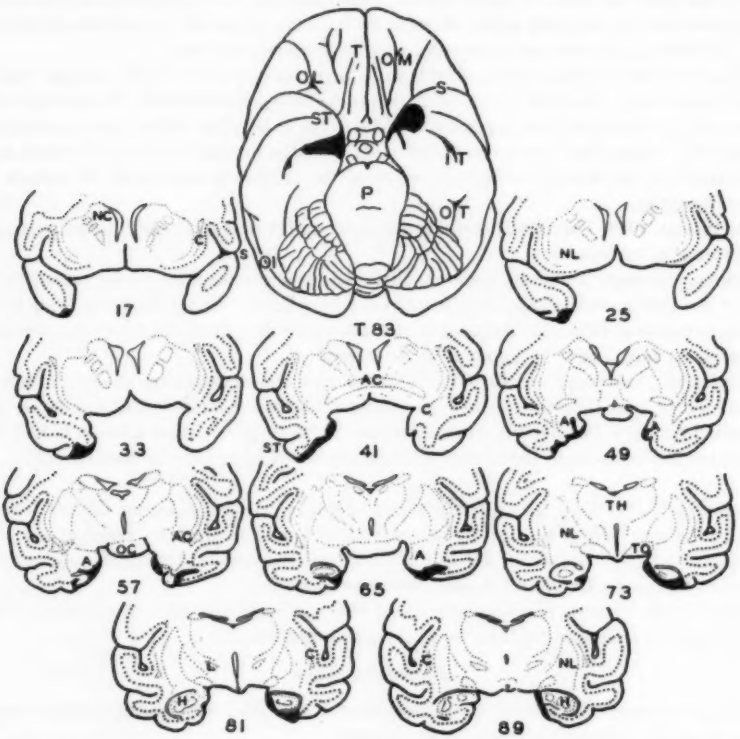

FIG. 6. Experiment 5. Sketches of the cerebral hemispheres and serial sections to show the lesions.

passes medially along the hippocampal fissure for about 0.5 cm. The deepest part of the lesion, situated laterally, is 0.3 cm. below the normal contour of the cortex.

In the serial sections of the brain the lesion commences at the tip of the superior temporal sulcus, extends medially to within 2 mm. of the incisura temporalis, damaging the basal part of the cortical amygdaloid nucleus. Slightly more caudally there is a linear injury in the amygdala passing through the lateral and accessory basal nuclei. The base of the complex is removed along with the parvicellular part of the basal amygdaloid nucleus, but the posterior third of the complex is intact. Only a small area of cortex is damaged between the hippocampal and rhinal fissures (Fig. 6).

Postoperative State

May 20, 1949. The animal is quite playful, hanging from the ceiling of its cage and curiously watching everything about it. It is so tame that it can be caught and held with one's bare hands. It responds normally to sensory stimuli, but even when molested shows no anger or rage reaction.

June 14, 1949. The monkey still can be fed from one's hand. It adopts peculiar positions, lying on its back and rolling around. Its interest in other monkeys is perverted; occasionally it picks a fight with other females, and frequently presents to males.

July 28, 1949. The animal's behavior remains unchanged. When presented a number of objects the edible ones are eaten and the others completely ignored.

August 9, 1949. The animal is not as tame as previously, and may snarl if molested. It will still take food from one's hand and allows itself to be petted. In the cage it has a tendency to adopt peculiar attitudes or postures which may have a sexual significance but the animal seems to have a more normal sexual activity now than formerly.

During the fall months the animal became less tame; it died on November 14, 1949, six months after its second operation.

Experiment 6. (M. T97) A timid female monkey; medial temporal lobe ablation sparing uncus and amygdaloid complex; superficial damage hippocampal gyrus; after operation no change in behavior.

This timid female macaque came under observation April 6, 1949 (weight 2.8 kg.). The animal had no abnormalities on neurological examination and reacted well to the various forms of stimuli.

Operations

On May 5, 1949 a left temporal craniotomy was performed and an area of cortex on the medial surface of the temporal lobe was ablated. The animal had no behavioral changes after this procedure. On May 10, 1949 a similar operation was carried out on the right temporal lobe.

Pathological Anatomy

Right hemisphere. The right lesion measures 1.8 x 0.8 cm. Its tip is 0.7 cm. posterior to the Sylvian fissure. Its medial border crosses to the hippocampal gyrus just posterior to the tip of the rhinal fissure. The lateral margin roughly parallels the superior temporal sulcus for approximately 1 cm., then turns medially near the level of the inferior temporal sulcus and passes across to the hippocampal gyrus. The lesion is relatively superficial at no point being more than 0.4 mm. below the surface of the cortex.

Examination of serial sections of the brain confirms the gross location of the lesion. At no point is the amygdala damaged and the hippocampal gyrus is injured only superficially in its lateral portion.

Left hemisphere. The lesion of the left temporal lobe is roughly quadrilateral measuring 1.2 x 1.1 cm. Its anterior extremity lies medial to the tip of the superior temporal sulcus and 0.6 cm. from the Sylvian fissure. The medial margin of the lesion follows the Sylvian fissure to the medial aspect of the lobe crossing the rhinal sulcus. The lateral margin passes just medial to the inferior temporal sulcus and ends 1.8 cm. caudal to the temporal pole. The lesion is superficial, being not more than 2–3 mm. below the contour of the cortex.

Serial sections of the cortex confirm the location of the lesion. Although the lesion lies just ventral to the amygdaloid nucleus, the latter is not damaged at any point. The cortex

between the rhinal and hippocampal sulci is removed and the hippocampus only slightly injured (Fig. 7).

Postoperative Status

May 5, 1949. The animal is wild and aggressive if molested. It bites if one approaches near it, so that gloves must be worn to catch it. The animal has normal sexual relations.

June 21, 1949. No tameness has developed. The animal appears to be wild and aggressive as before its operations. It shows signs of affection towards and interest in other monkeys. Its sexual activity remains normal.

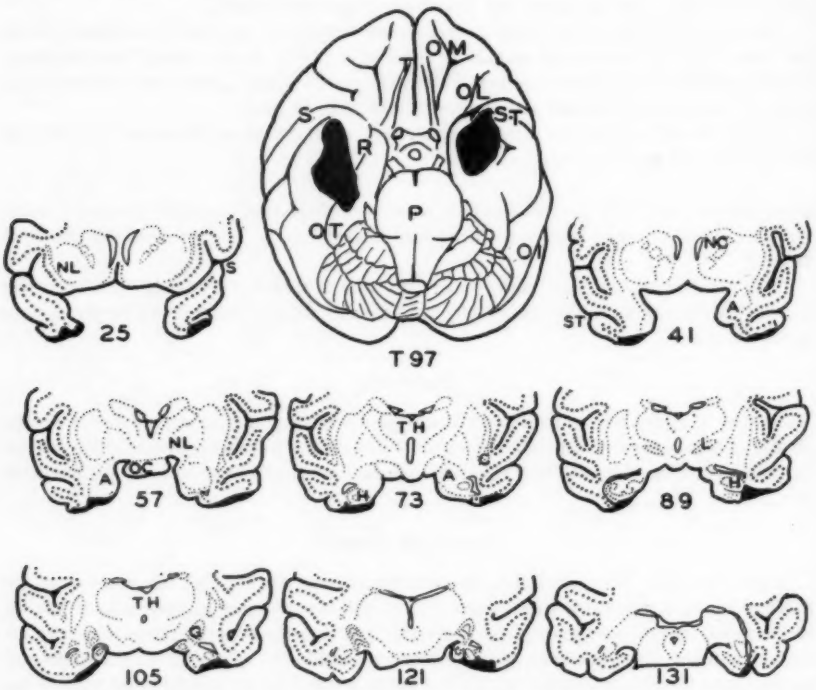

FIG. 7. Experiment 6. The extent of the lesions is indicated in the sketches of the hemispheres and serial sections.

July 28, 1949. When presented with the food tray, the animal picks up the food with the left hand without hesitation and eats. After a few minutes it picks up a match, puts it in its mouth but quickly throws it away. Then it throws the tray on the floor. When given a tray with food covered, it quickly discards the overlying cardboard and eats the food, all within five seconds.

During the next five months the animal was observed monthly. Its wild behavior was similar to that prior to its operations.

The animal was sacrificed on January 26, 1950, approximately 9 months after its second operation.

Experiment 7. (M. T9) A timid male macaque; bilateral lesions medial temporal lobe, involving inferior third of the amygdaloid complex; after operation tame and playful; when alone apathetic; tameness gradually decreased over 19 month period.

This male macaque came under observation May 15, 1949 (weight 2.3 kg.). The animal had no abnormalities on neurological examination and reacted well to all forms of stimuli.

Preoperative Status

Reaction to man. The monkey was quite apprehensive, crouching in the farthest corner from the observer.

Reaction to other animals. The animal took a normal interest in its cage mates, but did not appear to be aggressive. It had frequent sexual relationship, at times with other males. Occasionally it presented to other monkeys. When a cat was introduced in the cage the monkey inspected it but, when the cat clawed, lost all interest.

Reaction to food. The animal differentiated readily between edible and inedible objects.

Operations

On May 25, 1949 a left temporal craniotomy was performed and the uncus removed. The animal had no change in its behavior. On June 15, 1949 a similar procedure was carried out on the right side.

Pathological Anatomy

Right hemisphere. The lesion of the right temporal lobe lies on the medial aspect of the lobe measuring 1.2 cm. laterally and 1.0 cm. anteroposteriorly. It begins at the tip of the superior temporal sulcus and extends medially to within 2–3 mm. of the hippocampal fissure. Just caudal to the optic chiasm it passes laterally almost to the inferior temporal sulcus. The lesion is relatively superficial.

Examination of serial sections of the brain confirms the extent and location of the lesion. It damages superficially the cortical nucleus of the amygdaloid complex, and the basal third of the entire complex almost to its posterior extremity. The hippocampal cortex between the rhinal and hippocampal sulci is ablated, but the hippocampus only superficially damaged.

Left hemisphere. The lesion of the left temporal lobe lies along the Sylvian fissure and medial aspect of the lobe measuring 1.5 cm. laterally by 1.0 cm. anteroposteriorly. The medial margin of the lesion parallels the Sylvian fissure, the lateral swings outward at the level of the optic chiasm, crosses the inferior temporal sulcus and passing rostrally crosses the tip of the superior temporal sulcus to reach the margin of the Sylvian fissure. The lesion is superficial throughout.

The examination of serial sections of the temporal lobe confirms the gross location of the lesion. The cortical nucleus of the amygdaloid complex is damaged superficially but the inferior third of the entire complex has been removed almost to the hippocampus. The cortex of the hippocampal gyrus between the rhinal and hippocampal fissures has been ablated and the hippocampus injured (Fig. 8).

Postoperative Status

June 20, 1949. About this time a marked change is noted in the behavior of the animal. When the door of the cage is opened it remains quietly inside. Even if one's hand is inserted, the animal exhibits no terror or fear; in fact it seems to like to be patted on the head. Although it appears to perceive all modalities of sensation, it does not respond violently to painful stimuli.

June 30, 1949. The animal can be caught with one's bare hands. It has no fear of the observer; and will roll over on its back to play. A finger placed in its mouth is licked but not bitten. The monkey does not pay much attention to other monkeys; its sexual relations are diminished.

July 28, 1949. It usually sits motionless near the front of its cage hanging onto the bars with the hands. If the door is opened it may jump playfully but does not attempt to escape. It seems to have little interest in its environment or other monkeys. Although it has erec-

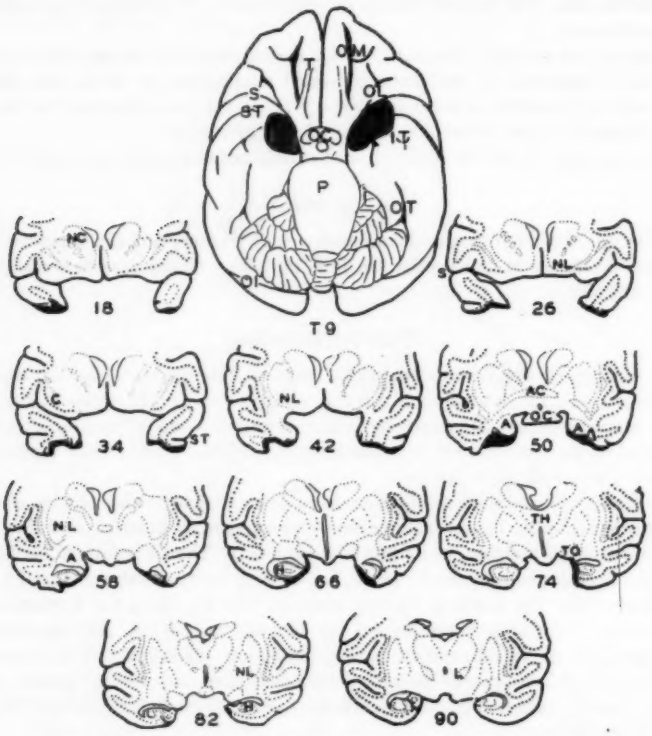

FIG. 8. Experiment 7. Sketches to show the extent of the lesion.

tions, it does not seek sexual relations. On one occasion it was seen to present in the presence of a male monkey.

August 9, 1949. When the test tray is given, the monkey picks up the food with its right hand but leaves the inedible objects. When presented with a tray with covered food, the animal throws away the covering cardboard with both hands, picks up the apple with the right hand and eats it.

November 3, 1949. During the past 3 months, the animal has been very tame and playful. At no time did it react violently, although it is quite active, and likes to be petted. Its sexual activity is normal, but it presents to males at times. The test tray of edible and inedible objects was responded to normally.

In the subsequent three months the animal was still very easy to handle, and could be caught without gloves. Usually it was playful but if provoked it snarled. The animal was quite active, in fact restless. It had frequent sexual intercourse.

The animal survived until December 7, 1950, nineteen months after its second operation, when it died of an acute gastro-intestinal disturbance. Until its death it was less wild than the usual monkey but in its later months could be caught only with gloves. Its sexual appetite appeared normal.

Experiment 8. (*M. T63*) *An aggressive female macaque monkey with six weeks old baby; bilateral lesions of medial temporal lobe involving the greater part of both amygdala; after operation apathetic and indifferent to offspring which died of neglect in 3 weeks; tame for 4 months, then more aggressive.*

This female macaque came under observation June 16, 1949 (weight 3.2 kg.). The animal had given birth to a baby on May 3, 1949, which it defended constantly, repelling any advances made by other monkeys or by the observer which might endanger the baby. The animal had no abnormalities on neurological examination.

Preoperative State

Reaction to man. The animal was restless and quite aggressive whenever the observer was about.

Reaction to other animals. The animal had very few sexual relationships and no homosexual interests. As mentioned previously the animal constantly guarded its six weeks old baby.

Reaction to food. The animal differentiated edible from inedible substances readily, usually grabbing the particles with the left hand.

Operations

On June 23, 1949 a left temporal craniotomy was performed and the cortex of the uncus and medial surface of the temporal lobe was removed. The animal appeared normal after this procedure. A similar ablation was carried out on the right side on June 29, 1949.

Pathological Anatomy

Right hemisphere. In the right temporal lobe the surgical lesion measures 0.8 cm. anteroposteriorly by 0.5 cm. laterally. It extends medially from the optic tracts into the inferior temporal gyrus. The tip of the temporal lobe has been removed, except for a strip of cortex on the inferior lip of the Sylvian fissure. The base of the lesion is 3 mm. below the contour of the brain.

An examination of serial sections of the brain confirms the location of the lesion. The pyriform cortex, the oral anterior amygdaloid area, and cortical amygdaloid nucleus remain but the greater part of the amygdaloid complex is destroyed. Posteriorly, a small portion of the lateral amygdaloid nucleus remains. The cortex between the hippocampal and rhinal fissures and the lateral portion of the hippocampal complex has been destroyed.

Left hemisphere. The lesion in the left side measures 1.0 cm. laterally and 0.7 cm. anteroposteriorly and is depressed 4 mm. below the normal contour of the brain. It extends medially from the superior temporal sulcus to the optic tract. In the center of the lesion is a small orifice, approximately 1 mm. in diameter connecting with the temporal horn of the lateral ventricle.

The examination of serial sections of the brain confirms the gross location of the lesion which has removed the amygdaloid complex entirely with perhaps the exception of the pyriform cortex and the anterior amygdaloid area. Posteriorly a portion of the amygdaloid

complex lying between the optic tract and fibers of the anterior commissure (medial amygdaloid nucleus, caudal anterior amygdaloid area) remains intact. The medial part of the hippocampus and overlying cortex has been ablated at the level of the posterior extremity of the rhinal sulcus (Fig. 9).

Postoperative State

June 30, 1949. In its cage the monkey sits motionless, seemingly indifferent to all its surroundings, even its baby. The latter, which clings to its mother, is ignored or brushed away. One may pick up the little monkey with its mother looking on apathetically. The monkey allows itself to be petted and handled without reaction.

July 7, 1949. When its cage door is opened the monkey walks out very slowly and approaches the observer without fear. It takes the food from the hand of the observer. The

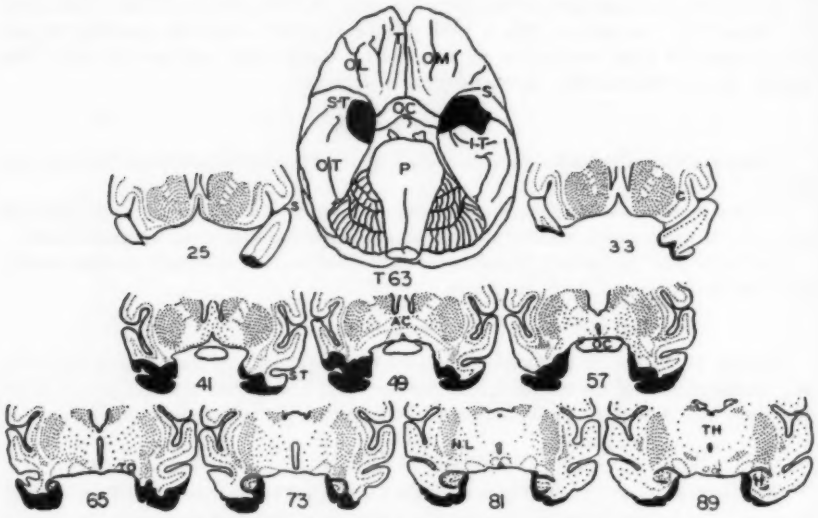

FIG. 9. Experiment 8. Sketches to show the extent of the lesion.

animal appears to perceive normally different sensory stimuli but to painful stimuli, like pinching, it withdraws but without anger. It pays little attention to other monkeys and seems to have diminished sexual activity. The baby can be removed from the cage without provoking any reaction on the part of its mother. At times, if the baby persists in its efforts to cling to its mother or to feed, the mother will knock it away or bite it. In fact, the baby is becoming covered with sores because of this treatment.

July 19, 1949. The baby monkey is taken from its mother because it is literally a mass of lacerations and bruises.

July 25, 1949. The baby died today. The mother, who has shown no signs of missing its offspring, occasionally snarls and shows its teeth when the observer approaches. It shows no interest in other monkeys and few signs of sexual activity. When a tray of edible and inedible objects is presented, the food is picked up without hesitation with the left hand. No attention is paid to the inedible items. When given the tray with the food covered, the cardboard is thrown away and the apple eaten without hesitation.

September 16, 1949. The animal is still indifferent socially and sexually to other monkeys. It is easily handled but may react angrily to painful stimuli. To other forms of stimulation, its response is normal. It has no signs of a vicarious appetite.

During October and November the animal gradually became more and more aggressive until it was impossible to catch it without gloves. The monkey never regained its original wildness. It was sacrificed on December 19, 1949, six months after its second operation.

Experiment 9. (M. T65) An aggressive male monkey; ablation of the medial surface of the temporal lobe including almost all of the right amygdaloid complex, the posterior two-thirds of the left complex and the hippocampal cortex; after operation no behavioral changes.

This male macaque came under observation June 17, 1949 (weight 3.1 kg.). The animal had no abnormalities on neurological examination and reacted well to all forms of stimulation.

Preoperative State

Reaction to man. The animal was quite aggressive and reacted violently to any molestation. Although generally it attempted to avoid the observer, at times the animal would act as if it were going to attack.

Reaction to other animals. The monkey was quite aggressive to other monkeys, both male and female. It was the largest monkey of the group and seemed to assume a certain regal attitude. It did not pick fleas from the fur of its cage-mates. The animal had normal heterosexual relationships. It reacted indifferently to the presence of a cat.

Reaction to food. The animal distinguished readily edible from inedible substances.

Operations

On June 24, 1949 a left temporal craniotomy was carried out and the uncus and amygdala removed by suction. The animal appeared normal after operation. A similar procedure was carried out on the right side June 29, 1949.

Pathological Anatomy

Right hemisphere. The right temporal pole is ablated extending from the medial surface of the lobe laterally for a distance of 1.5 cm. The excised area measures 7 mm. anteroposteriorly and the base of the ablation is depressed 1–2 mm. below the normal contour of the brain. The lesion extends medially to the margin of the optic nerve and passes posteriorly along the hippocampal gyrus. There is some cortex remaining medial to the rhinal fissure.

An examination of serial sections of the brain confirms the gross location of the lesion. Only a small portion of pyriform and dorsal part of the accessory basal amygdaloid nuclei remain. More posteriorly the ablation has removed the caudal half of the amygdaloid complex, the anterior part of the hippocampus and the cortex overlying it.

Left hemisphere. On the left side the lesion involves the medial portion of the temporal pole. It measures 1.4 cm. laterally and 0.7 cm. anteroposteriorly. There is some cortex remaining along the inferior lip of the Sylvian fissure at the temporal pole but posteriorly the ablation has removed practically all of the cortex along the rhinal fissure.

On examination of the serial sections it is seen that the anterior amygdaloid area is spared. A small portion of the accessory basal nucleus remains but the entire inferior part of the complex is ablated. A few cells of the magnocellular part of the basal amygdaloid nucleus and the dorsal part of the lateral nucleus are present but most of the posterior half of the amygdaloid complex is removed. The hippocampal cortex between the rhinal and hippocampal gyri is also severely damaged (Fig. 10).

Postoperative State

June 30, 1949. The animal is as wild and aggressive as before the operation.

July 7, 1949. In all respects, the monkey appears to be as before the operation. It is both socially and sexually active. Its appetite remains excellent.

August 17, 1949. The animal has been observed daily since its operations. Because its behavior is unchanged in any respect it is being sacrificed today.

Experiment 10. (M. TW). A rather timid male macaque; lesion left anterior perforated space sectioning anterior commissure, destroying the base of the septum and anterior hypothalamic nuclei; after operation no change in behavior.

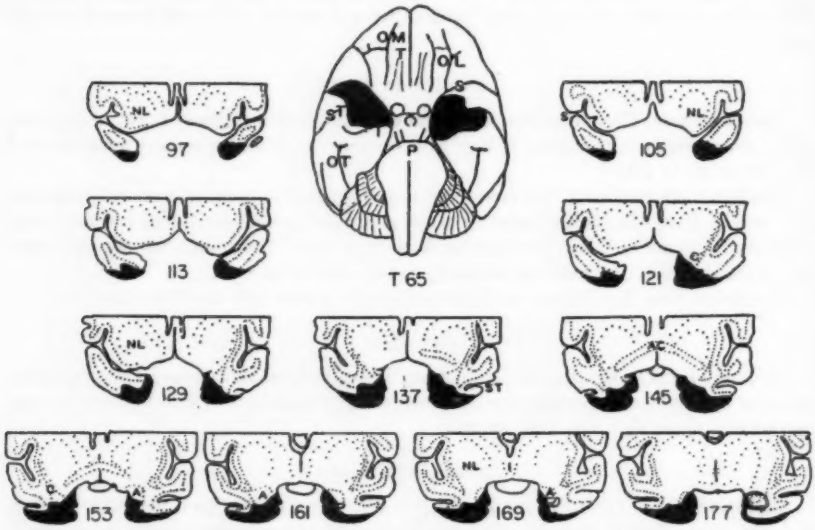

FIG. 10. Experiment 9. Sketches to show the extent of the lesion.

This male (weight 2.5 kg.) macaque came under observation July 7, 1949. The animal had no abnormalities on neurological examination and reacted normally to the various sensory stimuli.

Operation

On July 18, 1949 through a left temporal craniotomy, a lesion was made in the substantia perforata of the orbital surface of the frontal lobe. The animal had an uneventful convalescence.

Pathological Anatomy

The right cerebral hemisphere appears grossly normal; the left has an anteroposterior linear lesion just rostral to the optic chiasm.

Left hemisphere. The left hemisphere is slightly smaller than the right. A linear laceration measuring 1.3 cm. in length and 1 mm. in width passes from the medial aspect of the uncus to a point 3 mm. lateral to the tip of the superior temporal sulcus on the anterior margin of the left temporal pole.

On the left orbital surface there is a surgical laceration involving the anterior perforated space, extending laterally 1 cm. from the midline and anteroposteriorly 4 or 5 mm. from the Sylvian fissure.

Examination of serial sections confirms the gross location of the lesions. That on the left temporal pole is quite superficial and narrow, and does not extend into the white matter. The lesion of the orbital surface is more extensive. The rostrum of the corpus callosum is damaged by a small softening which extends beneath the corpus callosum to involve the cingular gyrus and the septum on both sides. On the left, the lesion extends laterally damaging a part of the head of the caudate nucleus and the basal part of the putamen. Further posteriorly the lesion destroys the base of the septum and the anterior commissure. The

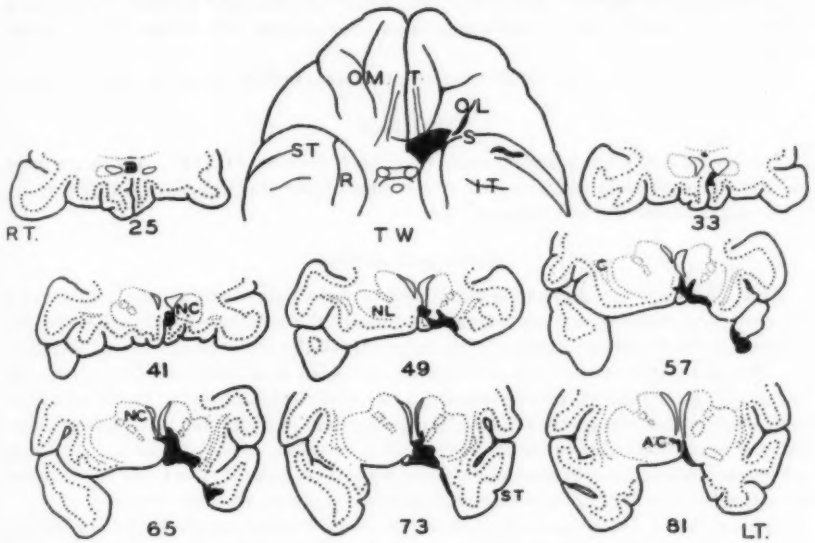

FIG. 11. Experiment 10. Sketches to show the extent of the lesion.

anterior hypothalamic nuclei just caudal to the septum are gliotic and probably largely degenerated (Fig. 11).

Postoperative State

July 28, 1949. The animal is so wild that it cannot be caught without gloves. Every move of the observer provokes an aggressive reaction on the part of the monkey. It seems to perceive and reacts to various sensory stimuli. To unpleasant olfactory stimulation the animal withdraws. It is quite aggressive towards other monkeys. Its sexual behavior seems to be normal.

August 11, 1949. The animal has developed no change in its social attitude and sexual activity since its operation. It was observed frequently in the following months but no behavioral changes were observed. Accordingly it was sacrificed on October 19, 1949.

Experiment 11. (*M. T102*) ***A normally aggressive male macaque monkey; ablation of the right uncus and amygdala; a small left temporal cortical ablation involving basal portion of middle***

medial amygdaloid complex; many degenerated cells of the basal accessory nucleus; after operation apathetic and without fear or aggressiveness.

This male macaque (weight 2.0 kg.) came under observation November 3, 1949. It had no abnormalities on neurological examination and reacted well to the various forms of stimuli.

Preoperative State

Reaction to man. The monkey was alert but somewhat apprehensive, following every movement of the observer. It would not accept food from the hand of the observer but withdrew to the far corner of the cage.

Reaction to other animals. The monkey was somewhat aggressive to other monkeys and particularly so toward cats. It had normal sexual relationships with various females of the colony.

Reaction to food. The animal differentiated edible from inedible items, ignoring the latter.

Operations

The animal had a left temporal craniotomy on November 30, 1949, an ablation being made of the uncus on the medial surface of the temporal lobe. On December 14, 1949 a similar lesion was made on the right temporal lobe.

Pathological Anatomy

Right hemisphere. Located on the medial aspect of the right temporal lobe is a lesion 1.5 cm. anteroposteriorly by 0.9 cm. laterally. Anteriorly the ablation extends to the Sylvian fissure, possibly involving slightly the posterior part of the orbito-frontal cortex. Medially it reaches to the optic chiasm. The cortex of the uncus has been almost completely removed leaving only a fragment of cortex measuring 3 mm. in diameter, medial to the rhinal sulcus.

In serial coronal sections of the brain the gross extent and location of the lesion is confirmed. The amygdaloid complex has been practically entirely removed. The lateral nucleus and magnocellular parts of the accessory basal nucleus are ablated. The lesion has removed the cortex between the rhinal and hippocampal sulci overlying the hippocampus.

Left hemisphere. The lesion on the left side measures 0.8 cm. laterally by 0.4 cm. anteroposteriorly. Its lateral margin lies 0.5 cm. from the Sylvian fissure, in the anterior part of the inferior temporal convolution and its medial border extends to the optic chiasm. The anterior part of the uncus is not damaged. Posterior to the ablation there remains a small amount of rhinencephalic cortex medial to the rhinal sulcus. The base of the lesion is from 1 to 3 mm. depressed in relation to the normal contour of the brain.

The examination of serial sections confirms the gross location of the lesion. The ablation however has involved little of the amygdaloid complex damaging only a small basal portion of the middle third of the medial amygdaloid complex and leaving intact almost all of the accessory and lateral nuclei. Although this nuclear complex remains, it is markedly degenerated containing many shrunken pale staining cells, especially in the basal accessory nucleus. The ablation removed the anterior part of the hippocampus and overlying cortex between the rhinal and hippocampal sulci (Fig. 12).

Postoperative State

December 30, 1949. The animal is very easy to handle and has lost completely its aggressiveness. It can be caught without gloves, and takes food from the hand of the observer. Even if one's fingers are held near its mouth, the monkey does not bite. When the door of the small

cage is opened, the monkey comes out very slowly. It shows no signs of affection towards other monkeys, but its sexual relations appear to be normal. It chooses only edible items when presented with a series of objects. Painful stimulation such as pinching the tail or different parts of the body provoke only withdrawal movements, but no snarling or signs of anger

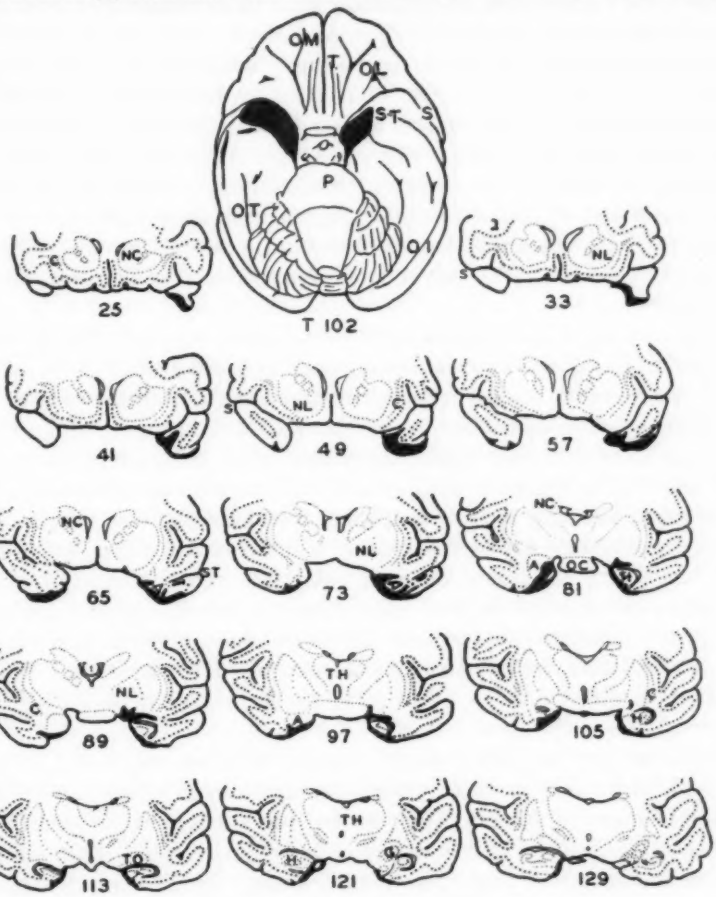

FIG. 12. Experiment 11. Sketches to show the extent of the lesion.

This marked tameness remained unchanged until January 3, 1950, when the animal developed a gastro-intestinal infection and died on January 5, 1950, one month after the second operation.

DISCUSSION

It is apparent that the animals in this series naturally fall into two categories—one, those which had no change in behavior after operation (Cases 1,

2, 6, 9 and 10), and the other, those which had alterations in behavior (Cases 3, 4, 5, 7, 8 and 11).

There are several points of interest in the first group which might be considered the control series. In practically all of these experiments there is extensive damage to the neocortex which gives rise to fibers of the anterior commissure, an observation which suggests that damage to this interhemispheral tract is not a factor in the behavioral alteration. Even greater confirmation of this hypothesis is the observation in Case 10 that the anterior commissure was completely severed without producing changes in conduct. The damage to the basal septal region and the anterior hypothalamus, including the ventromedial nucleus, is also of interest, since it suggests that these nuclei are not in the neuronal chain responsible for the changes in conduct. This does not confirm Wheatley's findings (13), but it is in agreement with the observations of Poirier (9).

The behavioral disturbances of the second group were quite similar and may be said to be changes in social behavior and personal attitude. The most common characteristic was an apathetic attitude of the operated monkey. When undisturbed, the animal sat on its haunches with drooping arms and head near the front of its cage apparently unaware of its entire environment. Klüver (6) and Poirier (9) have both emphasized this attitude following temporal lobe lesions including the rhinencephalon. Yet if the cage door were opened the animal would walk out to the observer with no apparent apprehension, allow itself to be petted or caught. Snarling, biting and aggressive traits were missing; strangely the opposite characteristics of shyness and fear seemed also to be in abeyance. Still the animal had not lost its emotional response of anger or rage for if its tail were pinched it would react violently or if it were more chronically irritated it would strike or knock away the annoying object—as the baby of Monkey T63 could attest.

Social activity was also changed. Signs of affection, manifested by picking at or licking other monkeys, were practically absent. Sexual activity was frankly diminished at least during the first two weeks after the operation. The tameness, fearlessness and asocial attitude gradually disappeared and by the fourth or fifth month after operation the monkey had practically regained its preoperative state. Two of the monkeys (T84 and T9) lived 18 and 19 months respectively after the procedure. Despite the fact that they were relatively easy to handle, terminally they were much less tame than immediately postoperatively.

In addition to the preceding features, the monkey with a baby (T63) showed what can be called a perverted maternal instinct. The signs of affection and devotion towards her youngster were supplanted by indifference and annoyance. If the baby sought the protection of her mother she was often rebuffed,

slapped or bitten. This was so serious that the infant's head became a mass of bruises and sores. The mother no longer made any effort to prevent the observer picking up and taking away her baby—in fact she seemed to be quite indifferent to the performance. This recalls the monkey that Klüver (4) reported, which after cuts between the frontal and temporal lobes, completely ignored its new born babies—even when offered them—and showed no resentment when they were removed.

It is obvious that the behavioral disturbances seen in these monkeys are not nearly so striking and diverse as those reported by Klüver and Bucy (7) following more complete temporal lobectomy. In fact, most of the components of the syndrome are conspicuous by their absence in all of our animals. Visual agnosia was not observed in any of the animals in this series, nor were the associated oral tendencies and hypermetamorphosis ever prominent features in any of the animals. Changes in sexual behavior and dietary habits were never well developed in any of the monkeys described. It may be that the immaturity of the animals in this series was a factor in the lack of hypersexuality such as characterized Klüver's animals. None of these monkeys was fully matured, although each displayed considerable sexual interest. In mature macaques the sexual aberrations might well be more prominent.

Even the changes in emotional behavior in these monkeys never reached the magnitude described by Klüver (6), whose temporal lobectomized monkey would approach every object without hesitation, be it cat, snake or a burning match, and endeavor to examine them orally.

It is of significance that the animals in this series that did show behavioral alterations had disturbances of the same type, although the degree of severity varied. This similarity in the changes produced by lesions of the medial surface of the temporal lobe suggests some common anatomical factor. If one compares the degree of behavioral change with the size and location of the anatomical lesions, one may conclude that, in this series, the injury to the temporal neocortex topically and quantitatively had no correlation with the magnitude of behavioral alteration (Table I). The same may be said regarding the injury to the hippocampus although damage to the fiber tracts, which cannot be adequately estimated, might vitiate this opinon. At first glance the damage to the amygdaloid complex seems to correlate with the degree of behavioral change but on a second look it is apparent that Monkey 65 challenges this conclusion. In this animal the left amygdaloid complex was practically entirely removed and on the right side the anterior and inferior portions of that mass were ablated. The monkey, however, exhibited no behavioral changes! Except for this experiment the thesis might be defended that damage to the amygdala was responsible for the alterations in conduct. But this case cast doubt on the hypothesis. And it cannot be discarded simply as an error due to mixing the brains for this

TABLE I

Experimental Procedures, Extent of Lesions and Clinical Results

EXPERIMENT AND MONKEY NUMBER	FIRST OPERATION	PERIOD OF OBSERVATION D—died S—sacrificed	DAMAGE TO ANIMAL OF						TAMENESS‡		
			Neocortex*		Amygdala†		Hippocampus†		Preoperative	Postoperative	
										0–6 mos.	Over 6 mos.
Exp. 1 (T 79)	Apr. 28, 1949	6 mos. (D)	++	++	±	±	±	+	0	0	–
Exp. 2 (T 73)	Apr. 29, 1949	6 mos. (S)	+++	++	0	±	+	++	+	+	–
Exp. 3 (T 85)	May 2, 1949	8 mos. (S)	++++	+++	++	++	++	++	0	++	±
Exp. 4 (T 84)	May 4, 1949	18 mos. (S)	+++	++++	++	++	++	++	0	+++	++
Exp. 5 (T 83)	May 5, 1949	6 mos. (D)	+	+	+	++	++	+	0	++	–
Exp. 6 (T 97)	May 5, 1949	9 mos. (S)	++++	+++	0	0	+	+	±	±	±
Exp. 7 (T 9)	May 25, 1949	19 mos. (D)	+	++	++	+	++	++	0	+++	++
Exp. 8 (T 63)	June 23, 1949	6 mos. (S)	++	+++	+++	+++	+++	+++	0	+++	–
Exp. 9 (T 65)	June 24, 1949	2 mos. (S)	++++	++++	++	+++	++	++	0	0	
Exp. 10 (T W)	July 18, 1949	3 mos. (S)	0	+	0	0	0	0	0	0	–
Exp. 11 (T 102)	Nov. 30, 1949	2 mos. (D)	++	+	+++	++	++	++	0	+++	–

* The surface area of neocortex damaged by each lesion was calculated. The largest zone of damage was graded as ++++; no injury as 0, and the intermediate grades were determined by dividing the difference into 4 equal units.

† The units represent approximately quarters of the whole complex.

‡ The units are clinical estimates of the degree of tameness.

monkey was sacrificed months before the other animals and its brain prepared by one of us (A. F. T.) before the other animals were killed. Perhaps there is an individual variation in the minimal amount of amygdaloid tissue necessary for its normal functioning. Certainly a considerable portion remained on the right side of Monkey 65. Yet other monkeys with even more residual amygdaloid tissue exhibited behavioral alterations. Thus we may only conclude that the amount of damage to the amygdaloid complex in general correlates fairly well with the degree of social and environmental (tameness) change of conduct. There are probably other factors influencing the emotional aspects of behavior.

The function of the temporal rhinencephalon has been studied in some detail recently. Much of the investigation has been carried out by stimulation of the structures and recording of the electrograms of various parts of the brain. Because these add little to our present problem, they will not be discussed further. For many years the amygdaloid nucleus and hippocampus have been suspected of having other than an olfactory role. Perhaps Papez (8) most clearly stated the proposition: "The hypothalamus, the anterior thalamic nuclei, the gyrus cinguli, the hippocampus and their interconnections constitute a harmonious mechanism which may elaborate the functions of central emotion, as well as participate in emotional expression" (8, p. 743). Klüver and Bucy's demonstration of the syndrome of bilateral temporal lobectomy in the monkey seemed to add support to this thesis. But in cats lesions of the olfactory tubercle, amygdala and hippocampus-fornix complex, seemed to induce rage reactions according to the acute experiments of Spiegel *et al.* (11) and the chronic experiments of Bard and Mountcastle (1). The latter concluded: "The area of the amygdala acts as a funnel through which inhibitory influences originating in the transitional cortex of the midline, in the neocortex and in the amygdala itself, exert a suppressing action on brain stem mechanisms" (1, p. 395). Perhaps the contradictory results are explicable on the basis that the extent of the lesions in the cat experiments are not quite comparable with those in the monkey or that the two species have different emotional mechanisms. The syndrome in the monkey resulting from ablation of the medial temporal lobe seems to be fairly well confirmed. In a brief abstract, Smith (10) states that lesions of the pyriform-amygdaloid-hippocampal complex caused decreased activity, loss of fear and defensive and offensive reactions and oral compulsive behavior. Anatomical controls were not given. Poirier studied a series of monkeys with bilateral temporal lobe lesions, most of which were rather extensive.

Poirier (9) found fragments of the syndrome described by Klüver and Bucy but he was impressed by the apathy and drowsiness exhibited by his animals. This led him to the view that the "amygdaloid area is one of the brain centers concerned with keeping the animal awake" (9, p. 235). We are in agreement

‡ The units are clinical estimates of the degree of tameness.

with Klüver (6) that at the present time attempts to break down the syndrome resulting from bilateral temporal lobectomy into neocortical and rhinencephalic components is premature.

There is evidence to suggest that the amygdala, hippocampus and neocortex play roles of varying importance in the production of the different components of the syndrome. On the basis of these experiments the amygdaloid complex may be said to produce usually the "taming" component of the syndrome, but localized ablations of other portions of the temporal lobe have not produced the other isolated components of the syndrome. Probably the temporal lobe functions are so interconnected and intergrated that a small lesion causes only a temporary breakdown of the general functioning which is quickly compensated. Whatever the explanation may be, the working of the temporal lobe is much more complex than the sum of a number of isolated functions carried out by individual anatomical structures.

SUMMARY

1. The behavioral changes in monkeys associated with bilateral lesions of the medial surface of the temporal lobe consisted of tameness, fearlessness and asocial behavior rendering the animal easy to handle and pet. Sexual activity was diminished initially. In one animal the maternal instinct was perverted. These changes gradually became less pronounced after the fourth or fifth month but some tameness persisted for as long as 19 months.

2. The behavioral changes correlated fairly well with the amount of damage to the amygdaloid complex—but there were exceptions to this thesis.

3. The function of the temporal rhinencephalon is discussed.

We wish to acknowledge the technical assistance of Miss Ruth Teague and Mr. Carl Jarrett in the histological preparation of the serial sections of these brains.

REFERENCES

1. BARD, P. AND MOUNTCASTLE, V. B.: Some forebrain mechanisms involved in expression of rage with special reference to suppression of angry behavior. Res. Pub. Assoc. Res. Nerv. & Ment. Dis. 1948, **27:** 362–404.
2. BROWN, S. AND SCHÄFER, E. A.: An investigation into the functions of the occipital and temporal lobes of the monkey's brain. Phil. Tr. Roy. Soc. London, 1888, **179B:** 303–327.
3. BUCY, P. C. AND KLÜVER, H.: Anatomic changes secondary to temporal lobectomy. Arch. Neurol. & Psychiat., 1940, **44:** 1142–1146.
4. KLÜVER, H.: Discussion of "Cybernetics. Circular causal and feedback mechanisms in biological and social systems." Trans. Seventh Conference. Josiah Macy, Jr. Foundation, New York, 1950, 226–228.
5. KLÜVER, H.: Functional differences between the occipital and temporal lobes with special reference to the interrelations of behavior and extracerebral mechanisms. In: Jeffress, L. A. (ed.): *Cerebral Mechanisms in Behavior*, Wiley and Sons, New York, 1951, 146–182.

6. KLÜVER, H.: Brain mechanisms and behavior with special reference to the rhinencephalon. J. Lancet, 1952: **72:** 567–577.
7. KLÜVER, H. AND BUCY, P. C.: "Psychic blindness" and other symptoms following bilateral temporal lobectomy in rhesus monkeys. Am. J. Physiol. 1937, **119:** 352–353.
8. PAPEZ, J. W.: A proposed mechanism of emotion. Arch. Neurol. & Psychiat., 1937, **38:** 725–743.
9. POIRIER, L. J.: Anatomical and experimental studies on the temporal pole of the macaque. J. Comp. Neurol. 1952, **96:** 209–248.
10. SMITH, W. K.: Non-olfactory functions of the pyriform-amygdaloid-hippocampal complex. Fed. Proc., 1950, **9:** 118.
11. SPIEGEL, E. A., MILLER, H. R. AND OPPENHEIMER, M. J.: Forebrain and rage reactions. J. Neurophysiol., 1940, **3:** 538–548.
12. THOMSON, A. F. AND WALKER, A. E.: Behavioral alterations following lesions of the medial surface of the temporal lobe. Folia psychiat. Neurol. Neurochir. neerl., 1950, **53:** 444–452.
13. WHEATLEY, M. D.: The hypothalamus and affective behavior in cats. A study of the effects of experimental lesions with anatomic correlations. Arch. Neurol. & Psychiat., 1944, **52:** 296–316.

FILTER PAPER ELECTROPHORESIS OF HUMAN HEMOGLOBINS WITH SPECIAL REFERENCE TO THE INCIDENCE AND CLINICAL SIGNIFICANCE OF HEMOGLOBIN C

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Since the discovery by Pauling (1) that normal and sickle cell hemoglobin differ in electrophoretic mobility, a reflection of molecular differences, the physical, chemical and biologic properties of these hemoglobins have been extensively studied. A number of techniques have been devised which materially assist in the clinical differentiation between sickle cell anemia and other conditions in which sicklemia may occur (2, 3, 4, 5, 6, 7). In the course of these developments, other abnormal hemoglobins were discovered which seem genetically determined and which at times are related to the occurrence of disease (8, 9). A standard terminology for the various types of human hemoglobin has been recommended by a group of workers in this field (10). Normal adult hemoglobin is designated as *hemoglobin A* and sickle cell hemoglobin as *hemoglobin S*. The two newly discovered abnormal hemoglobins are referred to as *hemoglobin C* and *hemoglobin D*. In addition to these four inherited types of hemoglobin, fetal hemoglobin or *hemoglobin F* is recognized as occurring normally in the red cells of the fetus and newborn, and is also demonstrable in the blood of patients with certain disease states (4). The term *sickle cell anemia* is well established to designate the clinical condition associated with homozygosity for S hemoglobin (S-S), whereas the individual who is heterozygous for the sickling character (S-A) is said to have the *sickle cell trait*. On the other hand, there is no generally accepted terminology for the clinical conditions associated with the various combinations of C hemoglobin. In this report the combination of C hemoglobin and normal hemoglobin will be referred to as the *C trait* (C-A). The disorder associated with the combination of sickle hemoglobin and C hemoglobin will be designated tentatively as *sickle cell-hemoglobin C disease* (*S-C*).

Although hemoglobins C and D have been recognized for some time, little is known concerning their occurrence or the clinical disorders with which they may be associated. The time, labor and expense involved in large scale electrophoretic studies have restricted advancement of knowledge in this field. Kaplan and his associates (11) and Itano and Neel (8) have published the findings in a few cases of the C trait and the C-S hemoglobin combination, and Neel,

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Itano and Lawrence (12) have drawn tentative conclusions regarding the probable incidence of C hemoglobin. The present paper is concerned with the description of a method of filter paper electrophoresis which has allowed us to gather a considerable volume of data regarding both the incidence and the clinical implications of this abnormal hemoglobin.

METHODS

Hemoglobin solutions were prepared from blood samples which had been prevented from clotting by the addition of citrate, oxalate or heparin. Red cells from at least one half ml. of

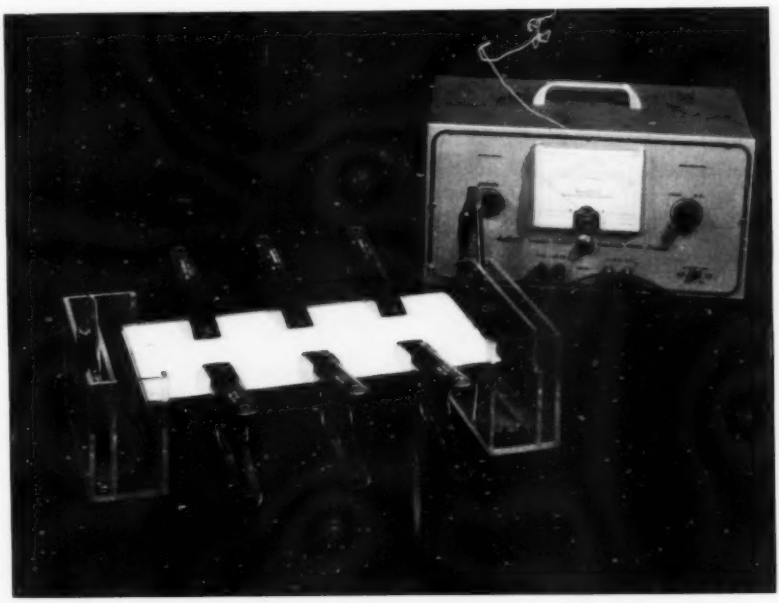

FIG. 1. The filter paper electrophoresis device used in this study. The Plexiglas buffer tanks have outside dimensions of 7½ by 3½ by 4 3/16 inches, with the electrode compartment having a depth of ½ inch. Other details are given in the text.

blood were washed with 0.85 per cent sodium chloride solution, and were lysed by the addition of approximately one volume of distilled water and one-half volume of toluene. Good results were obtained with final concentrations of hemoglobin ranging between 3 and 16 grams per 100 ml.

The *electrophoresis apparatus* used was similar in nature to that described by Kunkel and Tiselius (13), and more recently by Slater and Kunkel (14). It is shown in Figure 1. Whatman #1, #2, and #3 filter papers were used successfully, but the Whatman #3 seemed best for rapidity of migration and for ease of handling. The filter paper was cut into 5¾ x 12 inch sheets which increased in size to 6 x 12½ inches when wet with the buffer. The paper was supported between one-quarter inch plate glass sheets which were 6 x 12 inches. The

width of 6 inches was not critical, but when the length was less than 12 inches difficulty was encountered in maintaining a uniform rate of migration. Clamps of equal tension were essential to provide a uniform pressure over the whole glass plate surface. We found Hargrave Spring Clamps #2 most satisfactory for this purpose. The glass plates were supported at each end by buffer tanks made of $\frac{3}{16}$ inch plexiglass. A partition was placed to form a baffle between the electrode receptacle and the section which was in contact with the paper. This partition was notched to provide an electrolyte bridge of sufficiently low electrical resistance that only a small fraction of the applied electrical potential was lost. The inner lip of each

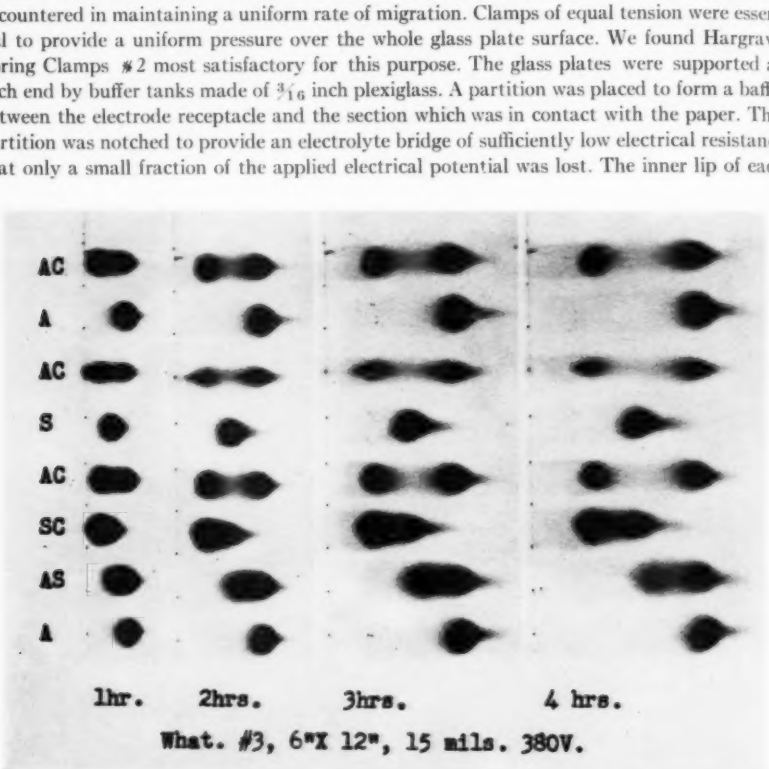

FIG. 2. The hourly illustration of migration was photographed while the electrophoresis was progressing, migration beginning from the dotted line. Dyeing or staining is not necessary for visualization using concentrations of hemoglobin greater than 3 per cent. Hemoglobin A is the fastest moving, hemoglobin S has intermediate mobility, and hemoglobin C migrates most slowly. Hemoglobin F was not present in concentration greater than 5 per cent of the total of any spot and is not demonstrable in this preparation.

buffer tank was incut sufficiently to receive the ends of the glass plates, thus providing an important advantage in that the buffer level could be raised above the level of the paper. This prevented drying of the paper and so eliminated the buffer concentration barrier mentioned by Slater and Kunkel (14). The power supply was a regulated voltage source with a range of 150 to 450 volts output with a current flow of up to 200 milliamperes. The voltage was adjusted so that the current flow through the electrophoresis unit was 15 milliamperes. Several such units could be operated simultaneously from a single power supply. The electrodes were of one $cm.^2$ platinum foil with mercury contact to the lead wire. During operation the anodal electrode became barbital coated with a resultant decrease of about one milliampere of current; however, this was of no consequence. A voltage drop of as little as 2 volts across one end of the paper was sufficient to produce uneven migration unless there was a

compensating fall in the opposite direction at the other end. This compensation was achieved by placing the buffer bridges at opposite ends of the buffer tanks. *Buffer* was veronal at a pH of 8.6 and of approximately 0.06 ionic strength. By reversing the electrodes at the end of each run the buffer could be used almost indefinitely.

From a Sahli pipette, 0.04 ml. of hemoglobin solution was applied to a line in the center of the paper which had been previously wet with buffer. The lateral edges of the glass plates were sealed with vaseline to prevent evaporation. Migration of the hemoglobin spots was evident within a very few minutes. Beginning separation of the various hemoglobin components was apparent within one hour, and by the end of two hours hemoglobin types A, S,

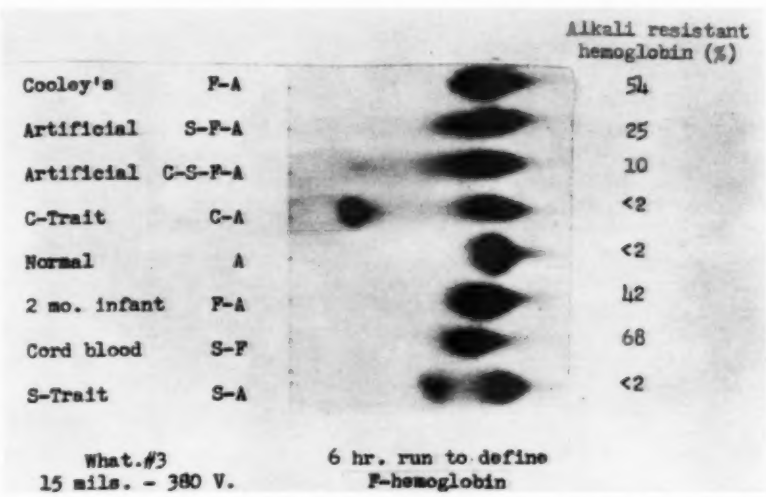

FIG. 3. Electrophoretic run using barbital buffer at pH 8.6, ionic strength approximately 0.06, and carried out for 6 hours in order to demonstrate F hemoglobin. The "artificial" preparations were mixtures of hemoglobin solutions from two sources.

and C could be identified (Fig. 2). By the end of four hours these hemoglobin types were widely separated, but F hemoglobin with a mobility very close to that of the normal was readily distinguished only after five or six hours (Fig. 3). Under the conditions of this method and the buffer used, hemoglobin C had the least mobility, less than one-half that of the fast moving A. Hemoglobin S had an intermediate rate while the F migrated only slightly more slowly than A. In each electrophoretic run hemoglobin of known abnormal composition was placed in the center of the paper as a reference spot.

The hemoglobin resistant to alkali denaturation was estimated by the method of Singer, Chernoff and Singer (4).

The minimum concentration of hemoglobin which would form a semi-solid gel when reduced, was determined by bubbling CO_2 through varied concentrations of hemoglobin (2).

RESULTS

A. Incidence of hemoglobin C in a clinic population

In an effort to determine the incidence of hemoglobin C in the population, a survey was conducted employing the electrophoresis technique which has

been described. Blood was obtained from one thousand patients who visited the five routine hematology laboratories of the out patient department of the Johns Hopkins Hospital on consecutive days. Since the population surveyed was from the out patient clinics, one would expect the results to be distorted to the extent that hemoglobin abnormalities produced disease. Thus, the incidence of sickle cell anemia should be higher in this group than in the general population. On the other hand there is no reason to believe that the sickle cell trait or the C trait should occur any more frequently in the hospital population than in a population at large since these hemoglobin abnormalities do not produce disease. The incidence of sickle cell trait in the group surveyed approximates that found in the general negro population in Baltimore (15). None of the individuals who were discovered to have C trait had symptoms which could be attributed to this abnormality and all had come to the hospital for unrelated reasons.

TABLE I

Occurrence of Abnormal Hemoglobins in Random Sampling of 1,000 Hospital Clinic Patients

RACE	NO. SAMPLED	S-S		S-A		S-C		C-A		C-C	
		No.	%	No.	%	No.	%	No.	%	No.	%
White	500	0	0	0	0	0	0	0	0	0	0
Negro	500	5	1	36	7.2	1	0.2	9	1.8	0	0

Results of the survey are shown in Table I. Of 500 white people screened no S or C hemoglobins were found, and since hemoglobin D has an electrophoretic mobility similar to S (9), this abnormality can also be excluded.

Of the 500 negroes screened nine were found to have the C trait and one the C-S hemoglobin combination, giving an incidence for C hemoglobin of 2 per cent. Individuals with sickle cell trait detected by electrophoretic examination have not as yet been further studied to determine if some might actually be instances of D trait. In addition to the nine individuals with C trait discovered in the survey five others have been encountered in other studies. These 14 persons have shown no regular hematologic or clinical abnormality. It is noteworthy that 13 of the 14 were female. There was anemia in only one, an infant with a history of poor nutrition and with no evidence of hemolysis; this infant is showing a good response to iron therapy. Blood smears of three of the subjects with C trait revealed no increase in target cells, while smears of four others showed a small but significant increase.

B. Clinical observations on individuals with sickle cell-hemoglobin C disease

In addition to the one person found on survey to have the C-S hemoglobin combination, we have studied six others with a like genetic disorder. The range in severity and clinical features has been striking.

CASE HISTORIES OF PATIENTS WITH SICKLE CELL-HEMOGLOBIN C DISEASE

Case #1. H. C. (JHH #628321), a 35 year old colored laborer was admitted on 3 occasions from 1938 to 1944 to government hospitals for investigation of recurrent hematuria which was demonstrated to be the result of bleeding from the left kidney. In 1938 an exploratory operation was performed after pyelographic studies had shown no abnormality; the left kidney appeared normal, but sickled red cells were seen in a section of lymph node. In 1944, because of recurrence of hematuria, a left nephrectomy was carried out, the kidney being described as normal. During these periods of observation, the spleen was described as enlarged. On one occasion it was reported that the red count was above 4 million, and on another that the hematocrit was 34 per cent. He was admitted to the Johns Hopkins Hospital in January 1953 for investigation of three generalized convulsive seizures which had occurred during the preceding six months. At that time he had slight scleral icterus, a left nephrectomy scar, and a spleen palpable at the level of the iliac crest. The remainder of the physical examination was not remarkable. Admission laboratory findings were: red cell count, 3.2 millions; hemoglobin 11.3 grams per 100 ml.; hematocrit 30 per cent; icterus index 20; white cell count 18,000 with 77 per cent granulocytes. More than half of the red cells were target cells. Serum bilirubin was 2.3 mg. per 100 ml. of which 1.1 mg. was indirect reacting, and urobilinogen was demonstrable in the urine to a dilution of 1:16. It was felt that the patient was experiencing a hemolytic episode. During the following week the hematocrit increased to 36 per cent, the serum bilirubin returned to normal, and the spleen decreased in size by one-half. Electroencephalogram disclosed an abnormal focus in the left parietal region. A cerebral arteriogram was normal. It was thought that he had had a vascular occlusion in the cerebral cortex, probably as a result of the sickling phenomenon. Since that time the patient has had two brief episodes of gross hematuria, the hematocrit has been 35 to 36 per cent, and the spleen has remained large. He was found by paper electrophoresis to have the S-C hemoglobin combination. The minimal concentration of hemoglobin which would form a semi-solid gel when reduced was 29 gm. per cent, whereas hemoglobin of a patient with sickle cell anemia gelled at 25 gm. per cent, and that of a patient with sickle cell trait gelled at 31 gm. per cent. Three and eight-tenths per cent of the hemoglobin was resistant to alkali denaturation, in contrast to the normal values of less than 2 per cent.

Case #2. S. R. (JHH #611651), a 16 year old colored school girl, was admitted to the hospital in July 1952 because of severe, acute tenderness and aching pain of the right knee and leg. The past history revealed that the patient had been well except for a similar but milder episode one year previously. Temperature elevation to 99.5 degrees F., tachycardia with many bursts of ventricular extrasystoles, a harsh apical systolic murmur, a palpable spleen and liver, and an acutely inflamed right knee with free joint fluid were noted. The hemoglobin was 12 gm. per 100 ml. blood, the hematocrit 35 per cent, and the icterus index was 5. Symptoms and evidence of the joint inflammation cleared during the following ten days and the patient was discharged from the hospital with the hematocrit remaining at 35 per cent. She was readmitted two weeks later with a history of passing grossly bloody urine for one week, but was otherwise asymptomatic. The hematocrit at this time was 19 per cent and the icterus index was 5. Urologic investigation revealed no abnormality but that the bleeding was from the left kidney. The patient was transfused with 1,500 ml. whole blood which raised the hematocrit to 37 per cent. The patient's red cells were demonstrated to sickle, and she was found to have sickle cell-hemoglobin C disease by paper electrophoresis. The alkali resistant fraction of the hemoglobin was 6 per cent. Blood smears stained with Wright's stain showed a preponderance of target cells (Fig. 4, a). The patient has remained well to the present time, the hematocrit has remained at 35 per cent, and the spleen is pal-

pable. Electrophoretic studies of the hemoglobin of members of this family show: mother C-A, 3 siblings S-A, 1 sibling S-C, one sibling C-A.

Case #3. G. W. (JHH #174945), a 20 year old colored woman was first admitted to the hospital in 1949 for delivery at term. There was no history of previous illness. During the sixth month of pregnancy she had been found to be anemic with a hematocrit of 23 per cent and icterus index of 10. There was no response of the anemia to iron therapy, and transfusion of 500 ml. of whole blood was used to increase the hematocrit to 28 per cent. Three days later, however, the hematocrit was 22 per cent. She delivered a normal infant. She was seen in the hematology clinic three weeks later where she was found to have a palpable spleen, hematocrit of 34 per cent with icterus index of 5, and bone marrow smears were suggestive of normoblastic hyperplasia. Sickling tests were positive and she was considered to have atypical sickle cell anemia because the habitus was not suggestive, there was no chronic and marked hemolytic anemia although there had been a hemolytic episode during pregnancy, and because of the palpable spleen at age sixteen. The patient has subsequently remained well with the hematocrit ranging from 32 to 35 per cent, and the spleen remaining large. Wright's

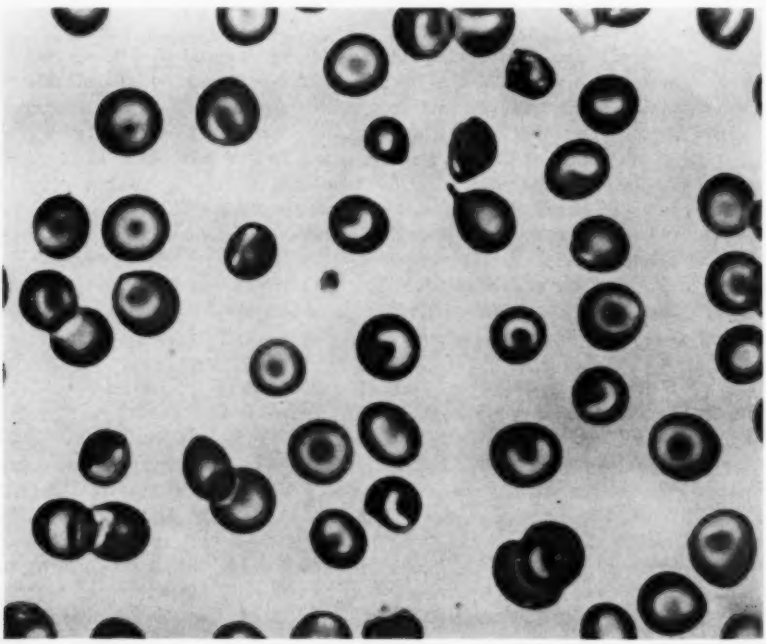

FIG. 4*a*

FIG. 4. Photomicrographs (× 1250) of blood smears of patients with sickle cell-hemoglobin C disease. (*a*) Wright's stained smear from Case 2 showing numerous target cells. (*b*) Wright's stained smear of blood of Case 4, showing the many target cells and the occurrence of sickled cells in a direct smear. (*c*) Blood smear from Case 4 shadowed with chromium. The small shadow cast by the target cells reflects their shallow depth.

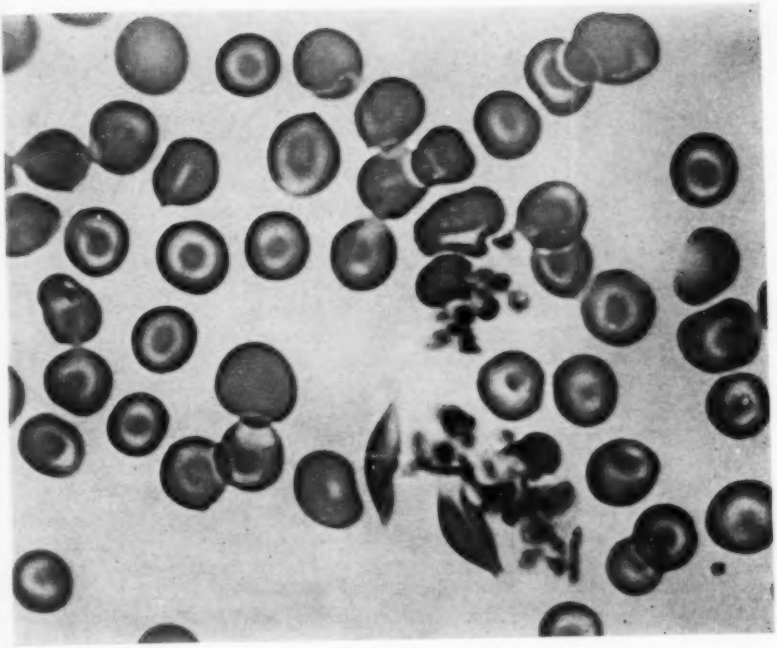

FIG. 4*b*

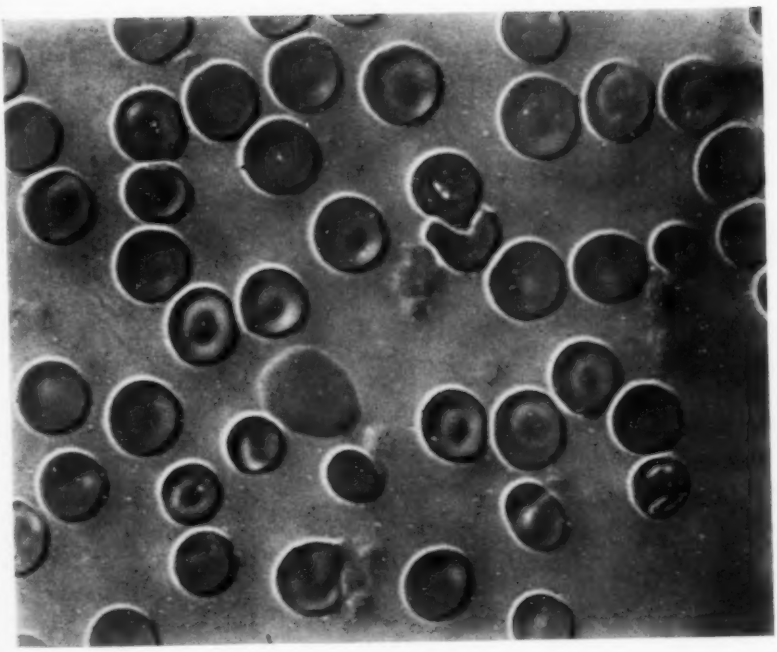

FIG. 4*c*

stained blood smears revealed a preponderance of target cells. A recent clinical diagnosis of sickle cell-hemoglobin C disease was substantiated by finding this hemoglobin combination by paper electrophoresis. The alkali resistant fraction of hemoglobin was 4.8 per cent.

Case #4. B. B. (JHH #257485), a 15 year old colored female was being followed in the pre-natal clinic when the survey to determine the incidence of hemoglobin C was being carried out. She was discovered to have sickle cell-hemoglobin C disease. She had been admitted to the pediatric service on two occasions as an infant, each time with evidence of lobar pneumonia and severe hemolytic anemia. The lowest hemoglobin determination on those occasions was 2.5 gm. per 100 ml. blood. Diagnoses of sickle cell anemia and possible rheumatic heart disease were made. She remained entirely well during the ensuing twelve years. Her pregnancy had been normal except for a mild anemia. Over several months increasing hematocrit values from 28 to 32 per cent, and finally to 35 per cent had been found. She was admitted to the obstetrical service in May 1953 in the thirty-sixth week of gestation because of two days of coughing, fever, and severe aching pains in the legs and knees. She presented evidence of mild bronchopneumonia which was controlled with penicillin. The sclerae were slightly icteric; the spleen was not palpable. She had none of the features of the sickle cell habitus, but the fingers were hyperextensible. During the next few days the severe leg pain persisted and there was a steady decline in the hematocrit from an admission value of 31 to 21 per cent. Simultaneously the icterus index increased from 5 to 12, and nucleated and polychromatophilic red cells appeared in the circulating blood. A blood smear stained with Wright's stain revealed a predominance of target cells but sickled cells were also seen (Fig. 4, b, c). The alkali resistant fraction of hemoglobin was determined to be 4.5 per cent. Transfusion of 500 ml. of whole blood was administered without reaction, and shortly thereafter the patient began labor and delivered spontaneously a normal male infant. Following delivery the leg pain abated considerably except in the left knee which had developed a hydrarthrosis. Cord blood of the infant revealed only ten sickled cells in an entire preparation with sodium metabisulfite, but by paper electrophoresis showed sickle and fetal hemoglobins. Sixty-eight per cent of this cord blood hemoglobin was resistant to alkali denaturation.

Case #5. R. R. a 13 year old colored boy was found to have sickle cell-hemoglobin C disease as a result of a survey conducted on the family of his sister (Case #2). His past history was completely negative but for the occasional occurrence of moderately severe leg cramps. His hematocrit was 39 per cent and the icterus index 17. An alkali resistant fraction of hemoglobin of 5 per cent was found. Target cells predominated on the Wright's stained blood smears. The only physical finding of note was the presence of an easily palpable spleen.

Case #6. T. N. (JHH #503965), a 15 year old colored girl has been followed in the out patient clinics since the age of five because of recurrent attacks of severe aching pains in the arms, legs, chest, and abdomen. These bouts have occurred on the average of five or six times a year, usually last three or four days, and are accompanied by fever and other systemic symptoms which have been suggestive of rheumatic fever. She was admitted in 1951 because of the most severe of such episodes. At that time pain was most intense over both tibiae, she had become nauseated and feverish, and developed cramping abdominal pain. On admission positive physical findings were: temperature of 102 degrees F., tenderness over the tibiae and the shoulders, tachycardia and harsh, systolic apical murmur and abdominal tenderness with slight muscular splinting in the right upper quadrant. Laboratory findings included red cell count of 4.1 millions, hematocrit of 31.9 per cent and icterus index of 15. White cells numbered 12,400 with 77 per cent granulocytes. The serum bilirubin was 4.6 mg. per 100 ml., of which 3.4 mg. were indirect reacting. Sickling tests were positive, and stained blood smears

revealed some polychromatophilia and a preponderance of target cells. During the next two weeks the pain subsided slowly, the temperature returned to a normal level, the serum bilirubin returned to normal, and the hematocrit decreased to 28 per cent. Since that time the patient has continued to have similar, but milder episodes of pain in the abdomen or extremities, and the hematocrit has been seen to range from 31 to 40 per cent. She had been considered to have atypical sickle cell anemia because of the relatively mild anemia, and because of the fact that the red cells of her mother could not be made to sickle using a variety of techniques. For this reason her blood was subjected to paper electrophoresis and she was proved to have sickle cell-hemoglobin C disease. In 1953 the spleen was palpable, and a predominance of target cells was again noted in the stained blood smear. The alkali resistant hemoglobin fraction of her blood was less than 2 per cent. The blood of the mother was examined and she was found to have the C trait.

Case #7. E. N. (JHH #459508), an 18 year old colored male, brother of another of our patients (Case #6), had been admitted to the pediatric service on three occasions in the past because of severe joint pains. The last of these admissions was in 1948, after which he remained asymptomatic until 1953. On each of the hospital admissions findings were essentially the same. He was a thin, acutely ill and feverish child with aching pain and tenderness over the knees, arms and on one occasion, the ribs. The spleen was not palpable. Hematocrit values ranged from 35 to 39 per cent, and on one occasion the serum bilirubin was 1.5 mg. per 100 ml., of which 0.7 mg. was indirect reacting. Sickling tests were positive, and numerous target cells were noted on stained blood smears. He was examined in the clinic on several occasions during 1951. On these occasions the physical examination was not remarkable, the hematocrit varied from 37 to 41 per cent, and the icterus index was 12. In 1953 he returned to the clinic because of severe leg pain. The spleen was not palpable; the hematocrit was 44 per cent. Five days later, however, when the leg pain had subsided, the spleen was easily palpable and tender. The hematocrit was 47 per cent, the icterus index was 13, and no reticulocytes were seen in 500 red cells counted. He had previously been considered to have "atypical" sickle cell anemia because the hematocrit was usually relatively high and because of the fact that his mother did not exhibit the sickling phenomenon. The alkali resistant component of his hemoglobin was less than 2 per cent, which is within normal limits. Recent study of his blood with paper electrophoresis has shown that he has sickle cell-hemoglobin C disease.

C. Demonstration of fetal hemoglobin by paper electrophoresis

By prolonging the time of electrophoresis it has been possible to demonstrate hemoglobin F, using 0.06 ionic strength barbital buffer at pH 8.6. Fetal hemoglobin was demonstrated in the blood of a normal infant of two months. Forty-two per cent of this infant's hemoglobin was resistant to alkali denaturation. Hemoglobin F was also demonstrated in the blood of a two year old child with thallasemia major, in normal cord blood, and in the cord blood of a child born to a patient with sickle cell-hemoglobin C disease. (The alkali resistant fraction of hemoglobin in these three patients was 54 per cent, 76 per cent, and 68 per cent, respectively.) In the latter patient hemoglobin S was also demonstrated (Fig. 3).

DISCUSSION

Detection of certain abnormal hemoglobins by electrophoresis is a well established procedure and the subject has been recently reviewed by Itano (5).

By use of this method it has not only been possible to differentiate with certainty between sickle cell anemia and sickle cell trait, but it has become apparent that certain other hemoglobin abnormalities exist (8, 9). Standard electrophoresis devices are not suited to mass surveys and for this reason it has not been possible to determine accurately the incidence of these newly discovered abnormal hemoglobins. The application of filter paper electrophoresis to the separation of the various hemoglobin combinations has provided a method which is accurate, rapid, and inexpensive. Spaet (16) using the method of Durrum (17), demonstrated that filter paper electrophoresis could be used for separating the human hemoglobins. In our experience the results have been much more satisfactory using the method described in this report than with the method of Durrum. With our method it is not only possible to demonstrate hemoglobins A, S, and C, but hemoglobin F can also be identified.

The results of the survey carried out in the Johns Hopkins Hospital suggest that hemoglobin C, like hemoglobin S, occurs predominantly in the negro. Up to the present time hemoglobin C has not been demonstrated in a white individual. In the negro the occurrence of hemoglobin C seems fairly frequent, with an incidence of 2 per cent in the colored population surveyed. The study of the individuals found to have the C trait confirms the benign nature of heterozygosity for this abnormal hemoglobin when combined with normal hemoglobin. While some of the individuals with this abnormality had as many as 10 per cent target cells on the blood smear, others had blood smears which could not be distinguished from normal. It is of interest that of 14 individuals who show the C trait, 13 are female. No other clinical or hematologic abnormalities were detected in these individuals.

Combination of hemoglobins C and S in an individual gives rise to a disorder which is in general much more mild than sickle cell anemia; however, we are more impressed than other authors with the evidences of severe disease which this combination can at times produce. While these patients usually have only mild anemia and no jaundice, they may develop hemolytic episodes during which jaundice appears and anemia becomes profound. In two of our patients such episodes have occurred during pregnancy. Pain in the extremities, so common in sickle cell anemia, appears to be less common in sickle cell-hemoglobin C disease, although in two of our patients such pain has been quite as severe as in sickle cell anemia. The severe episodes of abdominal pain which are so characteristic of sickle cell anemia do not appear to be a usual feature of this disease. It is noteworthy that two of our patients with this disorder had episodes of gross, unilateral hematuria. Goodwin and his associates (18) in 1950 reported a series of patients who had the sickling phenomenon and otherwise unexplained hematuria. It was thought that some of these patients had only the sickling trait; in retrospect, however, it is possible that some of these might

have had the S-C combination of hemoglobins. One of our patients displayed recurrent convulsive episodes, apparently attributable to an irritable focus in the cerebral cortex. It seems likely that the cortical lesion was the result of a vascular occlusion due to sickled erythrocytes. In other instances sicklemia has been accompanied by evidences of vascular occlusion in the brain, in the absence of significant anemia (19, 21). Six of our C-S patients have a palpable spleen, one of the six being an adult 35 years of age. In one patient the spleen was not palpable at the age of 9, but is now enlarged at age 13. Thus, there is a suggestion that splenomegaly not only persists into adult life in this disorder, but that it actually increases in degree. During hemolytic episodes the spleen may become transiently much larger. In all seven of the patients, blood smears stained with Wright's stain showed a preponderance of target cells, varying from approximately 60 per cent to almost 100 per cent. Although the mean cell diameter of these target cells on smear was larger than normal, shadowing with chromium revealed that the cell thickness was less than normal (Fig. 4).

Genetic studies by Neel and his associates (12) indicates that hemoglobin C is inherited as a Mendelian dominant character and is quite independent of the sickling gene. This hypothesis is given further support by the findings in the families which we have studied.

The clinical pattern of sickle cell-hemoglobin C disease as it is evolving is sufficiently distinct that it often can be recognized on clinical grounds. The relatively mild hemolytic anemia, the rarity of violent crises, the persistence of splenomegaly into adult life, and the presence in the blood smear of large numbers of target cells are characteristic of this disorder. In previous studies of sickle cell anemia in this hospital, patients were encountered who had a disorder which seemed to be intermediate between sickle cell trait and sickle cell anemia (19, 20). In a study of five of these cases of "atypical" sickle cell anemia, three were found to have sickle cell-hemoglobin C disease. It seems probable that further study of this group will disclose others that have this, or other abnormal hemoglobin combinations.

SUMMARY

A simple and inexpensive apparatus has been devised for the electrophoresis of hemoglobin solutions on filter paper. By the use of this device it is possible to recognize and separate hemoglobins A, S, C and F. The technique is well suited for application to mass surveys to determine the incidence of various abnormal hemoglobins. Such a survey has been conducted in the out patient clinics of the Johns Hopkins Hospital. In a study of the hemoglobins of 500 white persons, no instance of S, C or D hemoglobin was discovered. However, in 500 negroes surveyed, hemoglobin S was found to occur in 8.4 per cent and hemoglobin C in 2 per cent. The combination of hemoglobin S and hemoglobin

C in the same patient gives rise to a disease entity which on clinical grounds is readily differentiated from sickle cell anemia. Case histories of seven such patients are presented.

BIBLIOGRAPHY

1. Pauling, L., Itano, H. A., Singer, S. J. and Wells, I. C.: Sickle cell anemia, a molecular disease. Science 1949, **110:** 543.
2. Harris, J. W.: Studies on the destruction of red blood cells. VIII. Molecular orientation in sickle cell hemoglobin solutions. Proc. Soc. Exp. Biol. and Med. 1950, **75:** 197.
3. Ingbar, S. H. and Kass, E. H.: Sulfhydryl content of normal hemoglobin and hemoglobin in sickle cell anemia. Proc. Soc. Exper. Biol. and Med. 1951, **77:** 74.
4. Singer, K., Chernoff, A. I. and Singer, L.: Studies on abnormal hemoglobins. I. Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation. Blood 1951, **6:** 413.
5. Itano, H. A.: Human hemoglobin. Science 1953, **117:** 89.
6. Chernoff, A. I.: Immunologic studies of hemoglobin. I. The production of antihemoglobin sera and their immunologic characteristics. Blood 1953, **8:** 399.
7. Goodman, M. and Campbell, D. H.: Differences in antigenic specificity of human normal adult, fetal, and sickle cell anemia hemoglobins. Blood 1953, **8:** 422.
8. Itano, H. A. and Neel, J. V.: A new inherited abnormality of human hemoglobin. Proc. Nat. Acad. Sc. 1951, **36:** 613.
9. Itano, H. A.: A third abnormal hemoglobin associated with hereditary hemolytic anemia. Proc. Nat. Acad. Sc. 1951, **37:** 775.
10. Statement concerning a system of nomenclature for the varieties of human hemoglobin. Blood 1953, **8:** 386.
11. Kaplan, E., Zuelzer, W. W. and Neel, J. V.: A new inherited abnormality of hemoglobin and its interaction with sickle cell hemoglobin. Blood 1951, **6:** 1240.
12. Neel, J. V., Itano, H. A. and Lawrence, J. S.: Two cases of sickle cell disease presumably due to the combination of the genes for thalassemia and sickle cell hemoglobin. Blood 1953, **8:** 434.
13. Kunkel, H. G. and Tiselius, A.: Electrophoresis of proteins on filter paper. J. Gen. Physiol. 1952, **35:** 89.
14. Slater, R. J. and Kunkel, H. G.: Filter paper electrophoresis with special reference to urinary proteins. J. Lab. and Clin. Med. 1953, **41:** 619.
15. Wintrobe, M.: *Clinical Hematology*, Third edition. Lea and Febiger, 1949, page 502.
16. Spaet, T. H.: Identification of abnormal hemoglobins by means of paper electrophoresis. J. Lab. and Clin. Med. 1953, **41:** 6.
17. Durrum, E. L.: A microelectrophoretic and microiontophoretic technique. J. Am. Chem. Soc. 1950, **72:** 2943.
18. Goodwin, W. E., Alston, E. F. and Semans, J. H.: Hematuria and sickle cell disease: unexplained gross, unilateral, renal hematuria in negroes coincident with the blood sickling trait. J. Urol. 1950, **63:** 78.
19. Green, T. W. and Conley, C. L.: The occurrence of symptoms of sickle cell disease in the absence of persistent anemia. Ann. Int. Med. 1951, **34:** 849.
20. Green, T. W., Conley, C. L. and Berthrong, M.: The liver in sickle cell anemia. Bull. Johns Hopkins Hosp. 1953, **92:** 99.
21. Diggs, L. W. and Jones, R. S.: Clinico-pathologic conference. Am. J. Clin. Path. 1952, **22:** 1194.

STUDIES OF EXTRAHEPATIC BILIARY OBSTRUCTION IN THE WHITE MOUSE BY FLUORESCENCE MICROSCOPY*

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In these days of skillful medicine and surgery, it seems largely immaterial—insofar as the proper care of the patient is concerned—whether we know the intimate details of what happens in a liver subject to extrahepatic biliary obstruction. Nevertheless, the fascination of the problem remains, and will persist so long as there are major discrepancies in the findings and interpretations of able and earnest investigators. That there are such discrepancies, even the most devoted adherent of any school of thought will freely admit. To take one example, many distinguished physicians, with wide experience in diseases of the biliary tract, confidently believe that an obstructed liver routinely exhibits generalized and progressive dilatation, not only of the extra- and intrahepatic bile ducts, but of the entire bile canalicular system as well. Furthermore, it has been repeatedly claimed that such dilatation is accompanied by multiple ruptures of the bile canaliculi, with leakage of the bile into the blood and lymph. Yet there are others who seriously doubt or deny the occurrence of canalicular dilatation or rupture, or both, even in obstruction of long duration; and who would explain the sequelae of obstruction on quite different grounds.

It is this problem of the status of the bile canaliculi in extrahepatic obstruction which is the particular subject of the present report. One might well hesitate to attack a problem which has been so exhaustively studied, by a wide variety of methods, during the past half century and more. Our basis for doing so was that we had available a technic—that of fluorescence microscopy (1, 2)—which affords, in the living or freshly sacrificed animal, under normal and varying experimental conditions, more detailed and complete, three-dimensional pictures of the bile canaliculi than can possibly be achieved in fixed material by currently available methods. In the application of this technic to biliary obstruction we have been preceded by numerous investigators

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(3–13), some of whom, however, were not fully aware of the potential dangers of the technic itself. The most recent study along these lines is that of Hanzon (14), whose monograph appeared while this work was in progress. When it became evident that our findings did not agree with those of Hanzon in certain important respects, the work was continued in the hope of resolving some of these apparent discrepancies.

Our experiments have been conducted entirely upon white mice, with which we have had reasonably extensive experience—under normal and abnormal conditions—to serve as a background for proper evaluation and interpretation of the fluorescent images obtained in the obstructed animals. Observations of the bile canaliculi have been made at varying intervals following ligation and division of the extrahepatic bile ducts, with and without simultaneous removal of the gall bladder. The studies have been accomplished both *in vivo* and immediately after sacrifice of unanesthetized animals.

MATERIALS AND METHODS

The general procedures and equipment used have been developed in the course of earlier studies (15–19), and the characteristics of our fluorescence microscope—as currently assembled—have been described in detail elsewhere (17). All observations were made with the 6× monocular and, unless otherwise specified, with the 55× water immersion objective (without immersion cap and without the application of a cover slip to the surface of the liver). In each animal, the liver was exposed and stabilized as rapidly as possible, and the ventral surface of one or more lobes was promptly subjected to more or less continuous, sweeping survey at sharp focus. Particular emphasis was placed upon the character of the fluorescent images obtained during the very initial period of observations. All studies were accomplished with male specimens of the white mouse, obtained from Carworth Farms (strain CF1).

Fluorescein was employed in the form of its water-soluble sodium salt (uranine, Coleman and Bell Co., lot no. 480347). Vasoflavine was originally reported by Moses, Emery and Schlegel (20) as a purified fraction of thioflavine S, with almost ideal properties for visualization of blood vessels by Schlegel's technic (21, 22). Our sample was obtained from National Aniline Division, Allied Chemical and Dye Corp., lot no. 13763 (also designated as Erie Flavine S Purif.). The dyes were prepared as 1:500 solutions in mammalian Ringer's, and were routinely administered in a dosage of 30 mg. per kg.

Intravenous injection—commonly into the tail veins, in a few instances directly into the inferior vena cava—was adopted as the route of choice. Intraperitoneal injection, while highly satisfactory on other grounds, was rejected in order to avoid direct access of the dye solution to the surfaces of the liver which must subsequently be used for microscopic study; and possible puncture of a large, thin-walled, saccular dilatation of the extrahepatic ducts. Intravenous injection into the tail veins is at times partially or completely unsuccessful; and we have made it a practice—after completion of the microscopic observations—to inspect, under ultraviolet light, the site of injection and the pelvic tissues for local accumulations of the dye. Animals demonstrated in this way to have received inadequate intravenous dosage have been rejected from consideration. In anesthetized animals, exposure of the liver was routinely begun 5 minutes after completion of the dye injection; and unanesthetized animals were sacrificed (by crushing of the cervical cord) after the same interval.

Operative procedures: The mouse is anesthetized with chloral hydrate, using a 1 or 2% solution in 0.9% NaCl, dosage 45 or 60 mg. per 100 gm. of body weight, injected subcutaneously at 2 sites on the back, the areas being gently massaged after injection. Initial hypnosis is noted within from 5 to 10 minutes; a proper level of anesthesia is usually attained within 15 to 20 minutes. One hour following injection the animal is in general too reactive to permit accomplishment of a satisfactory operation in the upper abdomen, and is usually completely reactive at the end of c. 4 hours.

After securing the mouse in position by means of a pin through each paw, the abdomen is moistened with saline and an upper mid-line incision is made. The duodenum is exposed and retracted medially and caudally as far as possible; the intestines are then literally wrapped in the duodenal mesentery, and the right side of the abdomen is packed with a small, moist gauze sponge to maintain the duodenum in its displaced position. The lower edges of the liver are then gently pushed cephalad, and maintained in position by a small sponge placed against their under surfaces.

With the aid of a binocular loupe, the common duct is identified and completely exposed by downward traction on the duodenum. It is then carefully freed from the contiguous hepatic artery and portal vein by the use of a blunt-pointed nerve hook. A tie of either 5-0 or 6-0 black silk is passed around the duct, which is then firmly ligated. At this point two hazards must be kept in mind: 1. One must avoid 2 pancreatic ducts which enter the common duct from 3 to 5 mm. above the latter's entrance into the duodenum. It is desirable to place the first ligature on the common duct above the point where it begins its course through the head of the pancreas. 2. Excessive traction or handling of the duodenum may easily cause rupture of vessels in its mesentery, or interference with the hepatic artery itself. Most hemorrhage can best be controlled by pressure alone; clamping of vessels is usually disastrous in so small an animal.

The tie on the common duct, placed as described above, is left with long ends and is now used—instead of the duodenum—for traction purposes. The common duct is freed from surrounding structures as high toward the porta as possible. Here again, care must be exercised since the hepatic artery branches rather frequently as it approaches the liver, and these branches can easily be damaged or obstructed. A second tie of 5-0 or 6-0 silk is now passed around the common duct, and firm ligation is accomplished at the desired point, which is usually located 2 to 3 mm. below the entrance of the cystic into the common duct. Two accessory hepatic ducts—from the small omental lobes—are a constant finding, and it is frequently necessary to ligate them separately. After ligation of the upper tie, all traction on the common duct is released; it is then carefully elevated with a nerve hook, and completely severed between the ligatures. The gauze packs are removed, and the abdominal contents are returned to their proper positions, particular care being taken to insure that the duodenum is situated posteriorly.

If the gall bladder is to be simultaneously removed, it is clamped at the fundus with a small mosquito hemostat and delivered into the field. Following blunt separation of the avascular mesentery between the liver and the gall bladder, the cystic duct is completely exposed. A tie of 5-0 or 6-0 black silk is passed around the cystic duct, and ligated as close as possible to its origin from either the common hepatic duct or one of the separate ducts, as the case may be. The gall bladder is amputated distal to the single tie.

The peritoneum and muscles are approximated by a continuous stitch of 5-0 black silk on an atraumatic needle (Deknatel); larger suture materials or needles cause unnecessary adhesions. The skin is closed with a separate continuous stitch of 4-0 silk on a straight cutting needle. Finally, 1 or 2 ml. of 0.9% NaCl are administered subcutaneously in each flank. In keeping with the experience of others, it has proved unnecessary to take any

aseptic precautions in these abdominal operations upon white mice. The only effort which has been made, over and above ordinary cleanliness of instruments, etc., has been the sterilization—once for each operating day—of the pins placed through the paws of the mice for holding them in position on the operating board.

Maintenance of mice with biliary obstruction: All mice were kept singly or in pairs in large glass jars, provided with a wire-mesh platform covered with wood shavings. Food (Purina Laboratory Checkers) and water were available in excess at all times, and scrupulous cleanliness was maintained. When 2 mice were placed in a single jar, they were matched as to approximate states of health and levels of consciousness in order to avoid cannibalization. The temperature of the animal room was controlled at c. 29 to 30°C. Except for the saline injected subcutaneously immediately after operation (see above), no parenteral or supplemental drugs, special fluids, vitamins or antibiotics were administered unless otherwise specified. In many instances the basic diet was supplemented by milk (in various forms) added to the water, usually with beneficial effects upon the general condition of the animals. Food and water were allowed *ad lib.* up to the time of preparation for study.

Exposure and stabilization of the liver: The anesthetized animal (chloral hydrate, subcutaneous as before, 45 to 60 mg. per 100 gm.) is restrained in the supine position by placing a pin through the pad of each foot. The abdomen is moistened with saline, and a mid-line skin incision is made from the tip of the xiphoid process to a point just above the symphysis. The abdominal muscles and peritoneum are divided and opened in the mid-line, with care to avoid the superior and inferior epigastric vessels. Both halves of the abdominal wall are elevated and retracted laterally; 2 large bulldog clamps are applied on each side, and the skin and muscles are cut transversely between them. The falciform ligament of the liver is divided down to the inferior vena cava, and the tip of the xiphoid process is then amputated. Throughout the above procedures, the ventral surface of the liver is scrupulously avoided. The stomach is retracted caudally and, using 5-0 silk on an atraumatic needle, a suture is taken as high on the fundus as possible, and a second through the pylorus; this traction suture is then secured to one of the restraining pins of the lower extremities. By this means, the liver is to a great extent liberated from the influence of diaphragmatic movements. The liver is then supported and somewhat elevated on a saline-moistened flat plate, which is gently introduced under its dorsal aspect and rigidly secured to the side of the metal animal tray. In freshly sacrificed animals, after exposure of the liver, opening of the thoracic cavity and incision of the heart, the liver is similarly supported on a flat plate.

Following completion of the microscopic observations, each animal was subjected to gross autopsy, with particular reference to the status of the liver, extrahepatic ducts and gall bladder (and their contents), identification and status of the original ligatures, the possibility of the new development of fistulous tracts, infection, fat necrosis, and any evidence of portal or intestinal obstruction. Aspirated contents of the dilated ducts were routinely inspected under ultraviolet light, to determine the presence or absence of the fluorescent compounds employed. Liver tissue was usually fixed in 10% neutral formalin, and suitable blocks were embedded in paraffin and sectioned at 5 μ for staining in various ways (hematoxylin and eosin, iron hematoxylin, phosphotungstic acid hematoxylin and Mallory's stain). In many instances unstained sections (paraffin and frozen) were studied for the presence of pigment, etc.; and in selected animals frozen sections were stained for fat with Sudan III.

Injection studies of intrahepatic vessels: Most injections were carried out in anesthetized mice (chloral hydrate), the remainder immediately after sacrifice; a much more complete and uniform injection could be obtained in the living animal. Since the mice were invariably in a certain degree of shock following the trauma of opening, exposure and injection, with a resulting collapse of the peripheral sinusoidal circulation, the extent of the injection at the

surface of the liver could then almost always be easily and precisely controlled under the dissecting microscope.

The central veins were most frequently injected by cannulating the inferior vena cava below the renal veins with a polythene catheter, clamping the cava below the diaphragm and above the liver, and injecting Higgins blue waterproof ink (no. 4145, diluted 1:1 with normal saline, filtered). Occasionally, in sacrificed animals, the central veins were injected through the inferior vena cava above the diaphragm, after ligation of the cava below the liver. The portal system was always injected by cannulation of the superior mesenteric vein, followed by injection of Higgins black India ink (diluted 1:1 with normal saline, filtered). Whenever dual injections were performed, the hepatic veins were injected first, with an intact portal system. After study under the dissecting microscope, all specimens were placed in 10% neutral formalin. Following adequate fixation, the livers were sectioned freehand—in all representative planes—for further study. Treatment of the sections with the usual dehydrating and clearing agents resulted in marked or complete fading of the blue pigment.

Bilirubin determinations in serum and urine were accomplished by standard methods (23), using a Coleman Junior spectrophotometer at 540 mμ. Blood was drawn from the inferior vena cava, under chloral hydrate anesthesia, in animals used solely for this purpose. The amounts of serum obtainable in such small animals were necessarily limited (never exceeding 0.5 ml., and usually less), and did not permit accomplishment of duplicate determinations.

OBSERVATIONS

Studies with fluorescein

As discussed below, all other investigators who have studied biliary obstruction by fluorescence microscopy—in frogs and mammals other than the mouse—have worked exclusively with fluorescein, in the form of its water-soluble sodium salt (uranine). Under the circumstances, particularly since we were rather well acquainted with the behavior of the dye in the mouse under normal and other conditions, it was only natural that we should initiate our own studies of biliary obstruction with this same compound. The results were most discouraging, and have been repeatedly confirmed. Under our conditions, it has proved virtually impossible to achieve delineation of the bile canaliculi following acute obstruction of the extrahepatic ducts and the removal of the gall bladder. This is true even early in obstruction (within the first 24 hrs.; range of experiments, 9 hrs. to 25 days), irrespective of dosage (30 to 150 mg. per kg.) and route of administration of the dye (intravenous, subcutaneous, intraperitoneal, or a combination thereof), whether the liver is observed *in vivo* (chloral hydrate anesthesia) or immediately after sacrifice of unanesthetized animals. Furthermore, the dye frequently fails to appear in the cytoplasm of the hepatic cells at a detectable level. Except early in obstruction, we have seldom been able to detect the characteristic fluorescence of the dye in the contents of the extrahepatic ducts; and we can only interpret our findings to mean a more or less stubborn refusal, on the part of the obstructed mouse liver, to transfer significant amounts of fluorescein into the bile. It became quite evident that this dye was wholly unsuitable for our purposes; and search

for a more useful fluorescent compound led rather quickly to vasoflavine, with which the bile canaliculi can usually be delineated quite well at any stage of biliary obstruction.

Studies with vasoflavine

Before proceeding to a detailed description of the findings in the experimental animals, it will be necessary to review briefly important features of hepatic structure in the mammal, and of the fluorescent images normally obtained. Furthermore, under varying conditions (including more or less prolonged observation under the fluorescence microscope, ultraviolet radiation, anesthesia, anoxia, etc.), there may develop a wide variety of abnormal phenomena, which are in general highly repetitive in character. A thorough knowledge of these unusual pictures is essential in any attempt to determine such characteristic features of biliary obstruction as may be accessible to study by the methods employed.

As demonstrated by Elias (24–27), the mammalian liver exhibits a laminar structure, the laminae being typically one hepatic cell in thickness. When working with the fluorescence microscope, particularly at the higher magnifications, one is limited to the pictures obtained at and just below the surface of the liver. At the most superficial focus at which the bile canaliculi can be visualized, the picture is one of a more or less continuous polygonal meshwork—the so-called surface polygonal pattern (17, 19)—displayed in the plane of focus, and interpretable in terms of subcapsular laminae (17), which appear to be a general feature of the mammalian liver (14, 27). At the next deeper focus—the so-called first sub-surface level (17, 19)—the picture is one of a fairly regular alternation of sinusoids and laminae, with the canalicular meshworks exhibiting an approximately vertical orientation. The canaliculi routinely show multiple connections with those at the same and other levels, and it appears that the fundamental architecture of the bile canalicular system is that of a continuous, never-ending polygonal meshwork (17, 19, 25).

Following the injection of fluorescein or thioflavine S (the less purified form of vasoflavine), the normal sequence of events is as follows (17, 19): The dye appears promptly in the plasma, then in the hepatic cells and the bile canaliculi. At the peak of excretion, all canaliculi are sharply delineated at high fluorescent intensity, and all hepatic cells exhibit diffuse cytoplasmic staining at relatively low intensity. The nuclei are apparently routinely spared by the dye. From the peak of excretion to complete elimination from the liver, the sequence is essentially a progressive diminution in fluorescent intensity, achieved first in the hepatic cells, then (much later) in the canaliculi. As long as the canaliculi are delineated they persist as a continuous system, without detectable interruptions in their course, and maintain their identity as discrete tubes with essentially linear contours. They are on the whole significantly larger in caliber at the peak of excretion than during its early and late stages. Furthermore, in a number of instances, one may observe modest enlargements of the canaliculi at branching points, and occasional bars of the polygonal meshwork may exhibit more or less generalized increase in caliber to 2 or 3 times "normal" size. In our experience, vasoflavine gives pictures of canalicular delineation, etc. entirely equivalent to those obtained with thioflavine S.

The abnormalities which may develop in the liver in response to various insults, and which can be visualized by fluorescence microscopy, are described elsewhere (13-15, 17, 45). They include the following: "*beading*"—a more or less irregularly discontinuous rather than a continuous delineation of the bile canaliculi; "*tendril formation*"—the new development of short, delicate, blindly ending branches from the canaliculi; "*sprouting*"—literally a sprout-

ing of numerous, small vacuoles from the canaliculi, with which many of the vacuoles remain connected for a time by delicate, dye-laden channels of varying caliber; *intracellular vacuole formation*—the appearance within the cytoplasm of the hepatic cells of spherical vacuoles, varying in number, size and fluorescent intensity, and arising either as a sequel to "sprouting" or at times, so far as one can tell, independently thereof; *discontinuity of bile canaliculi*—a discontinuity which is more extensive than that observed in "beading", and is a frequent accompaniment of intracellular vacuole formation, but may develop in its absence; *imbibition cells*—hepatic cells which exhibit, in comparison with the hepatic cells in general, a significantly higher level of cytoplasmic fluorescence attributable to the dye, and so stand out in more or less bold relief in the laminae involved; *"turbidity"*—the development of a "turbid" appearance of the laminae, with generalized staining of the hepatic cells, and apparently a sign of cell death.

In control studies with thioflavine S and vasoflavine, all of the above abnormalities except "turbidity" have been encountered repeatedly in the mouse. When examined in normal animals immediately after sacrifice, the liver is typically free of aberrant phenomena at the very beginning of observations. However, they soon begin to appear, and increase in number and degree with time. When first observed *in vivo* under chloral hydrate anesthesia, the liver either may be virtually free of them, or may exhibit them in modest numbers. If absent, they soon begin to appear, and in any case they increase in number and degree with time. Inadvertent trauma (though seemingly slight) to the surface of the liver may result in the rather rapid development of more or less striking changes in the injured area. Therefore, one must obviously be cautious about attributing any of the usual abnormalities—which can be encountered under such a wide variety of circumstances—to the special conditions imposed in a particular experiment.

It should be emphasized that, in contrast to fluorescein, thioflavine S and vasoflavine are remarkably photosensitive; and the initially sharp pictures of the bile canaliculi rapidly decrease in fluorescent intensity and clarity when any microscopic field is held under continuous observation (17). However, the initial pictures are so highly informative that they leave little to be desired, and persist sufficiently long to permit very careful scrutiny and evaluation of all interesting features of a particular field. In some ways, the marked photosensitivity is a distinct advantage in that it gives the observer adequate warning of possible injurious effects, and distortion of the fluorescent images, by the ultraviolet light in the beam of the microscope.

In view of the character of the data to be presented below, it should be stated at this time that there appears to be no doubt of the completeness of biliary obstruction in the experimental animals. This is evident from the development of jaundice, with concomitant rise in serum bilirubin levels; from dilatation of the extrahepatic ducts; and from a careful autopsy of each animal, to rule out loosening or improper placement of the ligatures, the development of fistulous tracts, etc.

1. Common duct ligated and divided, gall bladder removed; in vivo observations under chloral hydrate anesthesia

A series of animals prepared in this way was observed *in vivo* for intervals from c. half an hour to 59 days following placement of the ligatures. A particular study was made of animals during the first 48 hours, on the assumption that the effects of obstruction supervene rather quickly. During this period, one might anticipate the development of characteristic features of biliary stasis,

essentially uncomplicated by the sequelae of more prolonged obstruction. Contrary to what one might expect, the findings in these early animals could not be differentiated in any respect from those in normal controls. Granting the occurrence of occasional abnormalities ("beading", "tendril formation", "sprouting", intracellular vacuole formation and imbibition cells), variable in frequency and degree, and granting their tendency to become progressively more prominent with time, there was not the slightest reason to interpret these non-specific abnormalities as characteristic of biliary obstruction. Except for these aberrant phenomena, the bile canaliculi were entirely within the normal range in caliber and character. There was no suggestion of rupture of the canaliculi, or of leakage of the dye—from canaliculi, imbibition cells or otherwise—into the sinusoids or perisinusoidal spaces.

In the animals with obstruction of longer duration, up to approximately 2 months, the findings were fundamentally the same as those described above. Granting the occurrence of occasional instances of limited, more or less generalized dilatation, the great bulk of the canaliculi were entirely normal in caliber and otherwise, so much so that one would hesitate to attribute any significance to the few exceptions. Abnormalities of the usual types were encountered as before, but again were excluded from consideration as forming no part of the picture of biliary obstruction *per se*. One as yet unexplained finding is the occasional occurrence of patchy "aggregates" of the dye, variable in size and contour, noted at or just below the surface of the liver. These "aggregates" were routinely delineated at high fluorescent intensity, and with rare exceptions were devoid of recognizable cellular structure.

In many instances, following completion of the *in vivo* observations, the animals were sacrificed and the same areas of the liver previously studied were promptly resurveyed at sharp focus, with no interference from respiratory and other movements. With few exceptions, there was no appreciable difference between the *in vivo* and postmortem pictures in so far as the bile canaliculi were concerned. More particularly, there was not the slightest suggestion that the canaliculi had undergone any generalized or localized alteration in caliber or character following death. Considering the smallness of the microscopic field and the large size of the area surveyed, it is not surprising that one occasionally encountered after sacrifice an interesting field which had been entirely missed *in vivo*. However, supplemental observations of this sort in no way altered the general conclusions drawn from study of the living animals.

The character and scope of the findings in such preparations are illustrated by the following protocol of one of the more interesting animals:

Mouse A54: common duct ligated and divided, gall bladder removed; 23 days later, under chloral hydrate anesthesia, vasoflavine intravenously; liver studied in vivo and after sacrifice.

Oct. 16: 19.0 gm.; operation under chloral hydrate (1% solution, 45 mg. per 100 gm. intraperitoneally); anesthesia good, procedure satisfactory; 4 ml. saline subcutaneously. *Oct.*

21: active, eating, no jaundice. *Oct. 28:* active, moderately jaundiced. *Oct. 29:* 16.5 gm., active, eating. *Nov. 7:* 17.0 gm., active, jaundice persists.

Nov. 8: 17.6 gm. *10:22 a.m.:* chloral hydrate (45 mg. per 100 gm., subcutaneously, 2 sites in back, areas massaged). *10:39:05–10:39:45:* 0.26 ml. vasoflavine 1:500 (30 mg. per kg.) intravenously (tail vein). *10:44:00:* exposure and stabilization of liver begun.

10:49:00–10:54:55, left lower lobe, ventral surface: Cytoplasm of hepatic cells routinely exhibits the dye at low intensity or somewhat higher, and there is widespread delineation of the bile canaliculi, on the whole at high intensity, in both the surface polygonal pattern and the underlying laminae. Numerous sinusoids exhibit a good level of circulatory activity. The great bulk of the canaliculi, with the exception of occasional instances of "sprouting" and rare stellate formations (see below), appear entirely normal in caliber and otherwise. Two exceptions merit particular comment: 1. A stellate aggregate of the dye at very high intensity, about the size of an hepatic cell, with multiple, unequivocally demonstrable connections with the surrounding polygonal meshwork of canaliculi, which are entirely normal in caliber. At the points of continuity the caliber diminishes rather gradually in some instances, more abruptly in others, to normal size. The central stellate figure extends right up to the sinusoidal margin, with no indication that it has ruptured into the sinusoid or perisinusoidal space (here not apparent). Its highly irregular contour is strongly against interpreting it as a cell territory. 2. A second stellate figure, not unlike the first, again with multiple, clearly demonstrable connections with the surrounding normal canalicular pattern, but not itself extending up to the sinusoidal margin. This looks like a dilatation at a branching point. In the course of survey several other analogous formations are encountered, more or less below the surface of the liver, but these cannot be visualized with sufficient sharpness for accurate interpretation. Instances of "sprouting" seem to increase significantly in numbers with time, and are frequently delineated at high or very high intensity. No unequivocal instance of intracellular vacuole formation is encountered, and there are no imbibition cells.

10:55:15–10:57:45, right upper lobe, ventral surface: The findings are fundamentally the same as in the left lower lobe, again with good circulatory activity in numerous sinusoids, but with definitely more numerous instances of "sprouting" and with a very occasional instance of intracellular vacuole formation (with or without apparent discontinuity of the adjacent canaliculi). A single stellate formation, entirely analogous to that described above under "2" in the left lower lobe, can be clearly visualized. Several others, more or less below the surface of the liver, cannot be clearly visualized, but suggest quite similar formations. If one discounts the "sprouting", the great bulk of the canaliculi appear to be entirely normal in caliber and otherwise. A few scattered, isolated imbibition cells (moderate intensity at best) are encountered toward the end of the period.

10:58:00: Sacrificed by opening both pleural cavities through the diaphragm; heart incised.

10:58:30–11:02:30, left lower lobe, ventral surface: The one significant finding, over and above those previously described, is a limited area of apparently unequivocal, more or less generalized dilatation of the canaliculi, involving several contiguous and interconnecting laminae. The picture comes into sharpest focus at the first sub-surface level. The fluorescent intensity of the canalicular contents is low, presumably attributable to partial extinction during the preceding survey. In these laminae the canaliculi are irregularly sacculated, and at intervals the caliber is reduced to normal or near normal size, to give a picture suggesting a string of sausages of differing lengths and diameters. In the most bulbous portions the caliber is as much as 7 to 9 times that of a normal canaliculus. Continuity with the surrounding polygonal pattern, of normal caliber, can be demonstrated—apparently unequivocally—at many points. The pictures are consistent with focal, more or less generalized dilatation in an otherwise normal canalicular meshwork. One can with confidence exclude, as a possible

basis for the pictures, unusual instances of "sprouting", intracellular vacuole formation or imbibition cells. A search for other similar foci of canalicular dilatation is unsuccessful.

11:02:45–11:07:30, right upper lobe, ventral surface: The findings are essentially the same as before, with the exception that instances of "sprouting" (highly variable in degree) are now quite numerous, and make one loathe to attach any significance to suggestive instances of canalicular dilatation when these are encountered. There are occasional instances of more or less marked intracellular vacuole formation, and a fair number of imbibition cells of low or moderate intensity.

Autopsy: The peritoneum is clean and free of any evidence of fat necrosis. The liver appears grossly normal in size and color. The proximal extrahepatic ducts are dilated, measuring 13 x 13 mm. and containing a clear fluid above a cloudy sediment. The aspirated fluid exhibits a characteristic vasoflavine fluorescence of low to moderate intensity. All ties are in place and secure. There is no interference with the pancreatic ducts, and the pancreas appears normal.

2. Common duct ligated and divided, gall bladder removed; observations in unanesthetized animals immediately after sacrifice

As a supplement to the *in vivo* observations, a group of unanesthetized animals was studied immediately after sacrifice, both during the first 24 hours and at varying intervals up to 64 days. In the series up to 27 days, the findings were essentially the same as those described above. Again, the great bulk of the bile canaliculi were entirely normal in caliber and otherwise, and there was no suggestion of canalicular rupture or leakage of the dye. The equivalence of the pictures obtained under the two sets of conditions would indicate that valid data upon the status of the bile canaliculi, etc. could be obtained with reasonable assurance in freshly sacrificed animals. This would avoid modifications in time relationships of the excretory process and other changes which might be attributable to the anesthesia. In the two mice which were sacrificed at longer intervals (50 and 64 days), and in which there appeared to be no technical blunder in the injection of the dye, etc., the bile canaliculi were delineated on the whole at relatively low fluorescent intensity, and in one case incompletely. This raises the possibility that, in late obstruction, the mouse liver may exhibit a reduced capacity to transfer vasoflavine to the bile canaliculi. Its more or less stubborn resistance to fluorescein, at all stages of the obstructive process, has already been discussed.

In one animal, a small amount of 50% glucose was injected into the common duct (following its ligation and division) via the gall bladder, which was then removed. Jaundice was first noticed about two weeks later, and had become quite severe by the 63rd day, when the animal was sacrificed following an injection of vasoflavine. The microscopic observations added nothing to what has already been said. The extrahepatic ducts were enormously dilated, their enlargement extending well into the substance of the liver.

3. Common duct ligated and divided, gall bladder left in situ; observations in vivo and after sacrifice

In animals so prepared, and acceptable on technical grounds, the liver was studied both *in vivo* and after sacrifice on the 22nd and 63rd days, at which time jaundice was intense (having been first noted on the 8th and 5th days respectively). With few exceptions, the bile canaliculi were within the normal range of size and character, and there was no indication that leaving the gall bladder *in situ* had significantly modified their status. The same was true in an animal sacrificed 4 days after operation, at which time jaundice was just beginning to appear. These animals did not afford any other information contributing to our understanding of the disease process.

Limitations imposed by the methods employed, and by the structural organization of the mouse liver

In working with the fluorescence microscope, at the relatively high magnification required for sharp visualization and accurate characterization of the bile canaliculi in mammals (in our case, the 55× objective was routinely employed), the observer is usually limited to scrutiny of the superficial and immediately underlying laminae of the liver. Although it is frequently possible to penetrate more deeply (17, 19), this involves progressive loss in clarity and fluorescent intensity; and in studies of the type reported here one derives practically all of his data from observations in the subcapsular laminae and in those at the first sub-surface level (see above). This is in itself a severe limitation, which cannot be overcome with currently available equipment; and we have had it constantly in mind.

The work had not progressed very far before we became acutely aware of the need for accurate information concerning the extent to which we regularly could or could not observe laminae in the 3 representative zones (centrilobular, midzonal and periportal) of the hepatic lobules. In routine surveys we did not encounter vessels recognizable as central veins or portal radicles; and from general structural considerations it seemed not unlikely that, on the whole, we were being denied access to laminae adjacent to both types of channel. In view of the tendency, in various types of hepatic disease, for parenchymal changes to be zonal in character and distribution, this would impose a still further limitation of large proportions. Under the circumstances, special studies were made of the parenchymal and vascular architecture of the mouse liver at and just below its surface. These were accomplished by vascular injections in anesthetized and freshly sacrificed specimens, and the findings were entirely consistent.

Injection via the hepatic veins: The blue injection mass is first clearly observed

in vessels deep in the substance of the liver, and approaching the surface more or less at right angles. The majority of these vessels will then branch into 2 or 3, occasionally 4 or 5, terminal central veins; and the remainder (c. one-third) will not. These unbranched vessels regularly approach the surface at right angles, the central veins—on the other hand—usually at an angle of c. 45°; and the latter are observed not infrequently to course at an angle of 10 or 15° toward the surface, at times almost parallel to it. As the injection progresses, small, delicate branches are delineated along the course and at the terminal ends of the central veins. At this stage, the portal vessels become strikingly counter-injected with blood, so that the portal limits of the lobules are very well demarcated. The injected central veins are always observed to be in fact centrally located in the lobules, with delicate sinusoids—filled with blood or with the blue injection mass—radiating toward the portal areas. With further advance of the injection, the sinusoids become progressively filled, seeming to spray or blossom from the ends and sides of the central veins until, at a later stage, the latter are no longer visible as anything more than rather dense condensations below the surface. Over the general surface of the liver (exclusive of its free margins, with which we are not here concerned), not a single central vein can be observed at the end of the injection, all having been obscured by filling of the sinusoids. The surface pattern now presents numerous, isolated, small, discrete, blue "puffs" or "blossoms", surrounded by clear regions in which the vessels (injected with blood) of the portal areas are well shown.

Injection via the portal vein: Much the same sequence of events is observed as following injection via the hepatic veins. The deep vessels divide into 2, 3 or 4 terminal branches, which usually course toward the surface at an angle of c. 45°. In general, the trajectory of the central veins is at a greater angle from the perpendicular than that of the portal radicles. As the injection proceeds, delicate sinusoids are first delineated from the sides and ends of the veins; then follows a complete sinusoidal injection, which wholly obscures the veins themselves. At the end of the injection, vessels larger than sinusoidal size—and filled with the black injection mass—are rarely observed at the surface of the liver. The latter then presents a repetitive polygonal pattern, with clear central areas surrounded by the black, "feathery" portal areas, which are everywhere continuous with each other. It is perhaps worth mentioning that areas of the liver in contact with other organs or structures always inject poorly, if at all, irrespective of the route of injection.

Studies of frozen and freehand sections of fixed and cleared injected specimens fully confirm the above observations, and leave no doubt that in the mouse the central veins and the small interlobular tributaries of the portal vein rarely achieve an immediately subcapsular position. Under the circum-

stances, we are forced to the conclusion that, under our conditions, we are being routinely denied access to the hepatic laminae adjacent to and surrounding the central veins and the portal areas. Although this limitation may not be as severe as it seems, we would appear to be largely confined to microscopic observation of laminae more or less midzonal in location. Some aspects of our findings are shown in the accompanying illustration (Fig. 1), which is admittedly far less satisfying than the vascular patterns as they are observed to develop, in three dimensions, in the original preparations.

Search of the literature reveals a paucity of discrete data upon vascular and other relationships at the surface of the mouse liver. However, a number of reports include general and specific information which has a very direct bearing upon our own problems. For the sake of compactness of presentation, these will be considered here rather than in the general discussion.

In his description of the liver surface in mammals, Pfuhl (28) expresses himself in very definite terms with respect to the central veins. In translation (pp. 409–410): "The hepatic veins are never in direct connection with the superficial vessels; the beginnings of the central veins are separated from the surface by at least a 'capillary length'." This generalization would be essentially in accord with our experience in the mouse. In speaking of the superficial lobular pattern, with its depressions where the interlobular regions reach the surface, he states (p. 409) that rather large branches of the portal vein, hepatic artery, lymphatic vessels and bile ducts course in these depressions; and that "Smallest branches of these vessels are present everywhere between capsule and hepatic parenchyma." On the other hand, he points out that "the lobular pattern is very dependent upon the turgor of the hepatic parenchyma and upon the current functional state of the liver."

Elias and Sokol (29) have recently described what they interpret as inversion or reversal of the lobular architecture in mammals, as a function of the prevailing blood pressures in the radicles of the portal and hepatic veins. In their report they include a series of photographs of the surface of the mouse liver, with a variety of patterns as established by the auto-injection of the sinusoids with blood under different experimental conditions. Although they do not specifically discuss this aspect of the problem, it would appear that their findings were on the whole consistent with ours; and that they in general likewise failed to observe —in an immediately subcapsular position—either central veins or small radicles of the portal vein.

The following statements by Hanzon (14, pp. 88–89), who has made extensive use of the rat in studies by intravital fluorescence microscopy, are particularly pertinent: "In the parenchyma under the superficial cellamina (Authors' note: our subcapsular laminae), the arrangement of the liver cells is the same as seen in a section through the organ. The sinusoids located between the cords converge towards the central veins which generally begin directly under the capsule . . . The interlobular vessels with accompanying formations, on the other hand, only very seldom reach as far as to the surface parts of the liver available for intravital investigation. Instead, the flow is seen to emerge from the depth in the convoluted sinusoids lying furthest away from the central veins . . . The liver cells, which are seen at the surface, to lie peripherally in the lobules, do not therefore occur in the far periphery, but those nearest to the interlobular vessels occur slightly below the surface. In exceptional cases an interlobular branch of the portal vein may be found near the surface with accompanying branches of the hepatic artery and the bile ducts."

It would appear that there are significant species differences among the mammals with respect to vascular and general architecture at and just below the surface of the liver. Our own experience certainly emphasizes the need for thorough acquaintance with one's experimental animal in studies with the fluorescence microscope, with its very limited range of penetration into the substance of the organ, particularly when the higher magnifications are required.

Utilizing transillumination technics with visible light, over a rather wide range of magnification, Loeffler and Nordmann (30), Wakim and Mann (31) and Knisely, Bloch and Warner (32) have carried out *in vivo* studies of the liver in a number of mammals, including mice. Their reports leave no doubt that they have clearly visualized the central veins, the interlobular tributaries of the portal vein, and the hepatic laminae immediately adjacent thereto. However, under their conditions, they were able—even at the highest magnifications employed—to penetrate far more deeply than we below the surface of the liver; and they provide no detailed information concerning the anatomical relationships at the surface itself. Furthermore, such transillumination studies are routinely performed at and near the free margins of the liver, which do not concern us here, and where structural organization differs in many respects from that of the liver as a whole.

Clinical course, development of jaundice, and serum bilirubin determinations

Before describing the clinical course in mice with extrahepatic biliary obstruction, it should be noted that—in our hands, at least—the postoperative mortality is high. Among 188 mice used for all purposes in this study, there were 6 operative and a total of 83 postoperative deaths from all causes. All of the latter group were subjected to ligation and division of the common duct; in 58 the gall bladder was likewise removed, in the other 25 it was left *in situ*. About half (41) of the animals died within the first 5 days (day 1, 11; day 2, 9; day 3, 10; day 4, 7; day 5, 4). The remainder died at irregular intervals scattered over the period from the 6th to the 69th postoperative day. There appeared to be no significant difference attributable to the removal or retention of the gall bladder. A total of 93 animals were used for study, and there are 6 long-term survivors at this time.

Unfortunately, we can provide definite information as to the cause of death in relatively few instances, either because it was not apparent at gross autopsy or because an autopsy was not performed. Fat necrosis was the presenting feature in 5 of the animals, obvious rupture of the dilated extrahepatic ducts in 4, pneumonia in 2, and gross infection (with frank pus) of the dilated ducts in 1. In the absence of more adequate data, we have been unwilling to attribute otherwise unexplained deaths to "shock" and so-called "hepatic failure."

Chloral hydrate was adopted for routine use in all operative procedures. We were undoubtedly encouraged in our choice by the following comment of Ravdin and Vars (33, p. 44): "It is well to remember that such a time-honored sedative as chloral hydrate has never been shown to cause hepatic injury."

After operation, a mouse will rather consistently lose several grams in

weight during the first 24 hours. Loss usually continues at a slower rate (1 or more grams per day) during the ensuing 4 or 5 days, after which the weight tends to be fairly well stabilized. Progressive loss after this period almost invariably is accompanied by death within a short time, as is the development of periorbital swelling. In animals which survive in good condition, there is seldom a return to the preoperative level. A significant gain in weight following stabilization is usually associated with re-establishment of continuity of the biliary tree. General appearance and activity may be quite misleading, for the obstructed mouse can—to a surprising degree—maintain apparent good health

TABLE I

Serum Bilirubin Levels in Mice at Varying Intervals Following Ligation and Division of the Common Duct and Removal of the Gall Bladder[1]

MOUSE NO.	DURATION OF OBSTRUCTION	SERUM BILIRUBIN TOTAL	MOUSE NO.	DURATION OF OBSTRUCTION	SERUM BILIRUBIN TOTAL
		mg. %			*mg. %*
A136	20 min.	Trace	A127	3 days	20.1
A137	90 min.	Trace	A128	4 days	18.0
A138	7 hr.	Trace	A131	4 days	24.5
A139	14 hr.	5.7	A134[2]	11 days	26.0
A129	17 hr.	10.1	A132	13 days	21.1
A125	18 hr.	6.3	A96[3]	20 days	25.5
A133	2 days	15.0	A98[1]	31 days	21.8
A126	2 days	20.0	A135[1]	44 days	30.0
A130	2 days	20.0	A89	67 days	32.0

[1] Gall bladder left *in situ* in mice nos. A98 and A135.
[2] Urine bilirubin: 11.0.
[3] Urine bilirubin: 14.0.

in the presence of extensive pathology. Most of the mice which were used for the microscopic studies remained active and alert, and continued to eat and drink satisfactorily throughout their clinical course.

In the obstructed mouse, jaundice of the ears and tail is seldom apparent—but may be suggestive—at 24 hours, but by the end of 48 hours is usually definite though slight in degree. The first indication of a rising serum bilirubin content is increased yellow coloration of the urine. Once it appears, jaundice becomes progressively more marked and persists at a high level. When the mice are maintained on the standard diet of Purina Laboratory Checkers, their stools are regularly brown in color. However, if the diet is altered to include only such foods as milk, cheese and cabbage, the stools then exhibit the characteristic clay color of biliary obstruction.

The level of serum bilirubin was determined in a relatively small series of animals, subjected to biliary obstruction for intervals varying from 20 minutes to 67 days (Table I). In the serum of normal controls, no more than traces of bilirubin could be detected by the methods employed.

Dilatation of extrahepatic bile ducts, their contents, and other aspects of gross pathology

Following obstruction, dilatation of the extrahepatic ducts may develop within the first 24 hours, and is always present after 48 hours or more. It increases in magnitude at a variably rapid rate, and may attain surprisingly large proportions. The size and character of the dilated sacs encountered in a selected group of animals are shown in the accompanying illustrations (Figs. 2–7). These are sufficiently representative to render additional illustrations unnecessary. The dilatation of the extrahepatic ducts appeared to be approximately equivalent irrespective of the presence of the gall bladder. When present, the latter was consistently dilated, as was the cystic duct, though never to a degree approaching that of the common and hepatic ducts.

In 21 mice with the gall bladder removed, the contents of the dilated sacs were aspirated, and were either colorless (animals 7, range 4–67 days) or were pigmented as follows: yellow (3, 2–23), green (4, 6–42), yellow-green (5, 9–59), brown-green (2, 64 and 67). In 9 mice with the gall bladder *in situ* (range, 4–66 days), aspirated fluid always exhibited a yellow, green or yellow-green color except in 1 animal (contents of ducts green, of gall bladder white). Vasoflavine (as demonstrated by its characteristic blue fluorescence) could customarily be detected—in variable concentration—in the duct contents early in obstruction (through the 2nd day). Beyond this time it was absent in the majority, questionable in some, present in the remainder. In obstruction of long duration, a high fluorescent intensity of the dye was found in only 3 animals, in each of which a biliary fistula could be clearly demonstrated.

In general, at any stage of biliary obstruction, the liver is surprisingly normal in size, appearance and consistency. With pronounced dilatation of the extrahepatic ducts, it is quite apparent that the intrahepatic ducts are likewise involved in the process. In the animals used for microscopic studies and serum bilirubin determinations, it was not unusual in early obstruction (within the first few days of operation) to find from 1 to several grossly recognizable areas of hepatic necrosis. Beyond this time, more or less extensive necrosis was observed in occasional animals scattered over the entire range of the experiments. However, this was not true in the majority, and we could only conclude that, in the mouse, grossly evident necrosis is not characteristic of biliary obstruction *per se*. The necroses occurring soon after operation could presumably be explained on the basis of involvement of finer branches of the hepatic artery in the operation itself. It would seem that those occurring later could also be explained on grounds other than biliary stasis, and might well be largely nutritional in origin.

Adhesions from the operation were usually minimal, and there was rarely gross evidence of local or generalized infection. There was nothing to suggest

portal or intestinal obstruction, even in the presence of tremendously dilated extrahepatic ducts. Instances of peptic ulcer formation were not recognized, but could easily have been missed; and there was nothing to indicate a hemorrhagic tendency in any of the animals. Recession of jaundice was usually associated with clearly demonstrable fistulous tracts, with re-establishment of more or less free drainage of the bile. Instances of fat necrosis were almost certainly attributable to trauma of the pancreas or actual obstruction of its ducts at the time of operation. In biliary stasis of long duration, the kidneys showed gross evidence of pathological change.

Histopathology

Although a careful gross autopsy was performed on all animals used for the fluorescence observations, no attempt was made to accomplish routine histopathological studies of the liver. These were confined to a selected group of 16 fairly representative animals, all of them subjected to ligation and division of the common duct and—with one exception—simultaneous removal of the gall bladder, and sacrificed from 1 to 67 days later. Such a limited series is admittedly inadequate to permit a detailed description of hepatic histopathology in biliary obstruction in the mouse. However, the observations indicate several important features of the process in this species, and justify brief comment.

Early in obstruction, the liver is devoid of recognizable lesions, save for the variable occurrence of areas of necrosis (see above). Later, dilatation of the major intrahepatic bile ducts is usually a more or less prominent feature, and may be quite marked. One sometimes encounters pictures of apparent "budding" of small ducts from the larger channels, the walls of which may exhibit papilliform, valve-like or quite irregular folds and elevations (Figs. 10, 11). Lumina of the ducts are regularly devoid of pigment accumulations. There is usually an appreciable increase in the amount of periductal connective tissue, and there may be an associated proliferation of the small ducts in the portal areas (see below).

Cellular infiltration of the periportal areas appears rather early in the process, and is uniformly present, though variable in degree, in 2 animals sacrificed on the 6th day. Beyond this time, it is present in all animals and in general becomes progressively more marked. On the whole, the cells are mononuclear in character. Proliferation of the small bile ducts—which are uniformly free of biliary thrombi—is at times quite obvious (Fig. 8), but is frequently slight or minimal, and may apparently be absent altogether. Such proliferation never even approaches the extraordinary extent to which it may occur in the rat, with invasion of the hepatic lobules and large-scale replacement of normal laminar structure (34). Appreciable amounts of connective tissue are usually demonstrable in areas of extensive cellular infiltration. In some instances,

multiple small, "empty" channels in the periportal areas are provided with delicate flat epithelium, and present an almost angiomatous appearance (Fig. 9).

In so far as hepatic laminar and cellular structure are concerned, parenchymal changes are for the most part minimal in extent. Lobular structure is well maintained, and there are no apparent regional or zonal changes. Preparations stained with Sudan III are almost uniformly negative for intracellular fat inclusions. In occasional animals, with obstruction of long duration, many of the Kupffer cells contain variable amounts of amorphous, yellow-brown pigment. In all animals, the bile canaliculi are uniformly devoid of recognizable biliary thrombi. More or less extensive observations upon the bile canaliculi indicate that, with few exceptions, they are entirely within the normal range in caliber and otherwise. However, we are unwilling to attach any particular significance to this finding, in view of the general untrustworthiness of such observations in routine autopsy material.

The presence of from 1 to several (or even more) areas of necrosis, usually small and in varying stages of development and resolution, is a fairly regular finding throughout the period covered by the series. Whether, in the mouse, such limited necrotic areas are characteristic of biliary obstruction *per se* could only be determined by study of more extensive material. Evidence of gross, active infection—in the form of sizable accumulations of polymorphonuclear cells in the duct lumina, etc.—is nowhere encountered. However, in several of the animals there is quite obvious invasion of the epithelium of the larger bile ducts by polymorphonuclears, which are likewise present in appreciable numbers in the areas of periportal infiltration.

It should be noted that our mice are usually from 6 to 10 weeks old at the time of operation. Under the circumstances, the changes described above must be interpreted as pathological in character, since changes associated with advanced age do not normally appear until quite late in this species (35).

DISCUSSION

This investigation has afforded us innumerable opportunities to observe the bile canaliculi, more or less completely delineated—with great clarity—in highly informative, three-dimensional pictures. These pictures have abundantly reinforced the conclusion—arrived at several years ago by Elias (25) from fixed preparations, and subsequently confirmed in this laboratory by fluorescence microscopy of living and freshly sacrificed animals (17)—that the fundamental architecture of the bile canalicular system in mammals is that of a continuous, never-ending, polygonal meshwork. The interconnections of the meshwork can be followed with relative ease in the fluorescent images; and leave little doubt that intercellular branches, ending blindly—characteristic of the amphibian liver—are of such infrequent occurrence in the mouse,

at least, both under normal conditions (17) and in biliary obstruction, as to be insignificant.

This study has likewise yielded numerous additional examples of the fact—originally described by Elias (25), and later confirmed (17)—that the bile canaliculi not infrequently course between the walls of the sinusoids and the adjacent hepatic laminae. A close relationship of some of the canaliculi to the sinusoidal walls has recently been re-discovered by Edlund and Hanzon (36). These authors have interpreted their findings in terms of a structural organization of the mammalian liver—i.e., liver columns with axial bile canaliculi, from which radiate blindly ending intercellular branches—which would appear to have been rendered untenable by the studies of Elias (24–27). Our own observations (17, 19, and this study) would firmly support the latter's concept of a laminar structure of the mammalian liver, the individual laminae being typically one hepatic cell in thickness.

It is obvious that the above structural relationships have a very important bearing upon the problems of biliary obstruction, and upon our findings in the present study. If the bile canalicular system is in fact largely continuous, a single canalicular rupture at any point could presumably release such back-pressure as might exist over a rather large portion of the system. Under the circumstances, the absence of generalized canalicular dilatation—even in obstruction of long duration—might be explained by ruptures so relatively few in number as virtually to defy detection by any available method, whether *in vivo* or in fixed material.

The canaliculi which course between the sinusoidal walls and the adjacent hepatic laminae—and so are not buttressed on all sides by the turgor of the hepatic cells—might well constitute points of structural weakness in the system, where ruptures could most readily occur. For this reason, we have been constantly alert to the possibility of detecting dilatation or rupture of the canaliculi in such locations, wholly without success. On the contrary, we have been consistently faced with canalicular pictures of such apparent normalcy that, had we not known of its existence, we would never have suspected biliary obstruction in the animals concerned.

In this discussion, no attempt will be made to review the vast literature which has developed upon extrahepatic biliary obstruction, as investigated by a wide variety of methods. Sturdy treatises have been written by others far more experienced than ourselves in these matters (37–41); and the alternative theories and interpretations of the available data are readily accessible in current textbooks and monographs. Our own studies have been accomplished with but a single species, and primarily by a single technic; and the applicability of our findings to other mammals and man, as well as their validity for the mouse itself, must await further investigation. Under the circumstances, we will confine ourselves to a review and critical evaluation of earlier reports upon

biliary obstruction—as investigated in living amphibia and mammals—by the technic of fluorescence microscopy, with which we are principally concerned.

Hartoch (3) was the first to study the liver *in vivo* following ligation of the common duct (the gall bladder was left intact). Although his descriptions and illustrations leave much to be desired, he found in a series of frogs that the bile canaliculi were delineated in a highly incomplete manner, at far lower intensity than normal, and only after a long delay. There was no dilatation of the canaliculi, even in obstruction of several days' duration; on the contrary, the canaliculi were of less than normal caliber throughout.

Franke and Sylla (6) reported briefly that in several frogs, following ligation of the common and cystic ducts, delineation of the bile canaliculi was more delayed and less uniform than in normal animals, and that imbibition cells were on the whole more prominent. They make no mention of having observed canalicular dilatation. On the other hand, in a subsequent study of mammals (7), without extrahepatic obstruction but subjected to poisoning with toluene-diamine, they noted highly irregular and quite striking dilatations of the bile canaliculi. In a subsequent presentation of these earlier findings, Franke (8) emphasizes the absence (with rare exceptions) of canalicular dilatation in early obstruction in frogs.

Ansorge (10) performed acute experiments in the frog, with ligation and division of the common duct near its entrance into the duodenum, followed by injection of fluorescein. She states that her findings were entirely consistent, as follows: Delineation of the bile canaliculi proceeded normally at first (up to 20 minutes), then was followed by marked dilatation of the canaliculi (up to 4–5 times normal caliber), primarily at points of branching. This condition persisted for 10 to 20 minutes, and was followed by "a return of the fluorescein from the canaliculi into the liver cells" (from her description, the development of numerous imbibition cells, accompanied by disappearance of the canaliculi). The dye was subsequently released from the liver cells into the blood stream. Despite further injection of fluorescein, delineation of the canaliculi could not again be achieved.

Markstahler (11) accomplished similar experiments in the rat, and found a different sequence of events, as follows: 15 minutes after intravenous injection of fluorescein, the bile canaliculi were well delineated, with numerous thickenings and with many vacuoles of the dye in the hepatic cells. After a further 10 minutes the canaliculi were surrounded by small vacuoles, which progressively increased in size. 52 minutes after the injection there was a sudden discharge of fluorescein from the vacuoles into the hepatic cells, which became completely filled with the dye (from her description, a rapid and widespread development of imbibition cells), and a concomitant disappearance of the bile canaliculi. She deduced, without having directly observed, a subsequent release of fluorescein in large amounts from the hepatic cells into the blood stream,

since disappearance of the imbibition cells—under continued observation—was not accompanied by redelineation of the canaliculi. The development of intracellular vacuole formation and of imbibition cells proceeded at different rates in different portions of the liver. Except for the "numerous thickenings" early in the process, she nowhere mentions dilatation or rupture of the bile canaliculi.

The report of Hirt, Ansorge and Markstahler (13) repeats, essentially verbatim, the earlier statements of Ansorge (10) and Markstahler (11). However, it includes an illustration (Fig. 6, p. 11) purporting to show the irregular, marked dilatation of the bile canaliculi in the frog following ligation of the common duct. On careful scrutiny, this figure fails to carry any conviction. A comparison with their Figure 2 (p. 6) will show that the canaliculi are for the most part within the normal range of caliber. The point of greatest dilatation in the course of an axial canaliculus would appear to be a so-called ampulla-like enlargement, a normal variant of the canalicular picture in the frog, and again—on the basis of our own experience (15)—within the normal range (although at the upper limit) of size. Pictures essentially equivalent to their illustration, including fusiform or spherical dilatations at the distal ends of intercellular branches of the axial canaliculi, have been repeatedly encountered in frogs without biliary obstruction (15).

Taken as a whole, the studies of Ansorge, Markstahler and Hirt are subject to the general criticism that they unwittingly concerned themselves in large measure with abnormal phenomena ("sprouting", intracellular vacuole formation, imbibition cells, etc.), which they interpreted as features of the normal mechanism of biliary excretion in both frog and rat (15, 17, 19). Under the circumstances, the validity of their findings and interpretations in animals with biliary obstruction is open to most serious question.

In a discussion of his earlier findings, Grafflin (16) noted that in preliminary experiments with extrahepatic biliary obstruction in the frog he had encountered very irregular, and at times bizarre, patterns of the bile canaliculi; and that he had discontinued the investigation when it became apparent that the frog was a poor experimental animal for such studies. The experiments were few in number, left much to be desired on technical grounds, and were never published. On critical scrutiny, it became apparent that the unusual findings could not be attributed to biliary obstruction *per se*.

Hanzon (14), in his *in vivo* studies of fluorescein (uranine) excretion in rats under Numal (aprobarbital, N. N. R.) anesthesia, worked exclusively within the first 36 hours following blockage of the common bile duct. He proceeded on the assumption (p. 228) that "probably all the changes in the bile secretion that can appear in biliary stasis are manifested during that space of time." After obstruction, the pressure in the ducts rose rather rapidly, and attained a maximum within 8 to 15 minutes (mean maximal pressure in 35 rats, 12.8

mm. of mercury). He states (p. 230) that in a given animal "the maximum was invariably the same under standard conditions for intravital microscopy for up to 5 hours. The pressure itself was unaffected by uranin secretion, only the rate at which it was attained being affected." His findings in early biliary stasis (from 0 to 35 minutes) are best given in his own words (pp. 230–234), which are quoted *in extenso* since his monograph may not be readily available to many readers of this report.

"During the increase of the pressure in the bile ducts, the uranin-filled bile capillaries ... were seen to become slightly wider and the fluorescence intensity was accentuated ... (Fig. 70). Here and there ... isolated, very sparse vacuoles appeared with strongly fluorescent uranin in connection with the bile capillaries ... Otherwise the picture of uranin elimination was unchanged ...

"Simultaneously with a maximal pressure in the bile ducts, or a few minutes earlier, ... a change occurred. Suddenly concentrated uranin was seen flowing in one or several sinusoids in many places in the liver (Figs. 71, 72). It was quite evident that the uranin accompanied the flow of blood towards the central vein where it was gradually diluted ... The liver cells along the sinusoids in which this flow occurred took up uranin which made them more markedly fluorescent than the other cells.

"The source of these uranin flows often lay in the far periphery of the lobules, being invisible at the liver surface, but were often also observable near the centre. Occasionally the flow was quite distinctly seen to come from a small strongly uranin-fluorescent vacuole or widened bile capillary branch. As a rule, the flow came from isolated, or small groups of, strongly uranin-fluorescent liver cells ... These uranin streams constitute a leakage from the bile capillaries to the blood, either between liver cells or through them ...

"When the bile flow was stopped early after the uranin injection, and the pressure rapidly increased, a greater number of leakages took place than when the stoppage was made later ... At a normal flow of bile, a smaller number of leakages is required for the outflow of bile from the bile ducts.

"Round the source of a leakage, occasionally an increasing number of cells were seen to become strongly uranin-fluorescent, and through them all violent quantities of concentrated uranin were seen to pass into the sinusoids in all directions (Fig. 73). At other times, only isolated injured liver cells with leakages were observed, though in greater quantities so that, on an average, one per lobule was to be found at the surface of the organ. In rare instances, only one or two leakages were seen in the whole examination area.

"Gradually after 15–30 minutes, the leakage through the cells would cease, while the injured cell would be visible for a longer time owing to its stronger uranin fluorescence. Instead, other leakages appeared in other places. This might be due to the swelling of the cells described in Chapter VII, which prevents the afflux from the bile capillaries to the injured cells.

"In no case was a decrease registrable in the bile duct pressure when considerable leakages occurred ...

"Apart from the above-mentioned changes, the picture of the uranin secretion was throughout quite normal. Most of the liver cells apparently secreted the uranin in a normal manner to the bile capillaries. The uranin content in the liver cells and later in the bile capillaries gradually decreased exactly as usual ..." (Authors' note: The above statements refer to experiments on 32 rats in which "a standard dose of uranin was first given intravenously, and 0–60 minutes later the efflux from the bile duct was stopped.")

"In five other experiments uranin was administered intravenously only 20–35 minutes after the biliary outflow had been stopped, and when the pressure had been at a maximum in the bile ducts from 5–20 minutes. The secretion picture of the substance became quite normal, as well as the secretion rate . . . When the bile capillaries had been filled with concentrated uranin also the leakages to the blood appeared as described above."

As quoted above, Hanzon tells a highly dramatic story—based upon *in vivo* observations and so particularly appealing—of the early sequelae of biliary obstruction. These include generalized dilatation of the bile canaliculi, ruptures of the canaliculi, increased permeability of the hepatic cells, and leakage of large amounts of fluorescein (and so presumably of bile) from the canaliculi, between and through the hepatic cells, into the blood stream. The occurrence of such phenomena has been proposed by others, using similar and different methods, but never in so persuasive a manner. We would like to share in the excitement, but frankly cannot, for reasons which we believe are sound. Some of these derive from our own repeated and consistent failure to confirm any of the phenomena in question as characteristic of biliary obstruction. These we regard as less important, and would gladly yield to convincing positive evidence from another quarter. More important are various features of Hanzon's own experiments which make it extremely difficult for us to concur in the validity of his findings and interpretations.

In the first place, his documentary fluorescence microphotographs (Fig. 70)—purporting to illustrate normal v. "widened" bile canaliculi in biliary stasis—are in our judgment entirely unconvincing. From our own experience, we would regard the caliber of the canaliculi in both photographs as within the range of the normal. In the second place, two of the major phenomena which he presents as characteristic features of early biliary obstruction—namely, "strongly uranin-fluorescent liver cells" (imbibition cells) and leakage of fluorescein in large amounts into the sinusoids—are described by Hanzon in an earlier chapter of his monograph as sequelae of more or less severe injury to the liver by ultraviolet radiation, alone or in combination with biliary stasis. From a careful study of his two separate presentations, it is almost impossible to avoid the conclusion that what Hanzon regards as characteristic features of early obstruction are in truth, from his own data, rather to be attributed primarily to severe injury by the ultraviolet light in the incident beam of his microscope.

He has observed, and we agree, that imbibition cells are a highly repetitive phenomenon, developing under such a wide variety of conditions that they seem to be "a general reaction of the cells to injury" (14, p. 193). In his experiments, they appear to develop more surely when radiation is combined with biliary stasis than with radiation alone. In our own experiments with obstructed animals, they are not present nor do they develop in unusual numbers during the initial period of observations; and we have not the slightest

reason to regard them as a characteristic feature of biliary obstruction *per se*. As for Hanzon's more or less massive leakages of fluorescein into the sinusoids, these have not yet been observed in this laboratory under any conditions. His photographs leave no doubt that he is dealing with a real phenomenon; but he can hardly justify it as characteristic of biliary obstruction when he has already described it, in entirely equivalent terms, as a sequel of severe injury to the liver by ultraviolet radiation.

Hanzon's experiments upon later stages of biliary obstruction, from 2 to 36 hours, require but brief comment. Like ourselves in the mouse, he encountered in the rat progressively reduced capacity on the part of the liver to transfer fluorescein into the bile canaliculi in detectable amounts. There was also partial or complete failure of the dye to appear in the cytoplasm of the hepatic cells. He again is inclined to attribute the appearance of imbibition cells to injury by the biliary stasis, and with this we would not agree. He observed no leakages of fluorescein into the sinusoids, but did note in some animals unusual areas—variable in number and size—which he interpreted as probably representing necrotic foci.

Some years ago it was pointed out (15) that all available observations, *in vivo*, upon the normal mechanism of fluorescein excretion by the liver agreed in offering no objective evidence for the existence of the frequently hypothesized potential lymph or tissue spaces between the hepatic sinusoids and the hepatic cells. *A priori*, it would seem that the method of fluorescence microscopy might offer an unusual opportunity for the visualization of these perisinusoidal spaces of MacGillavry (42) and Disse (43). Under the circumstances, in all subsequent studies—both under normal and under a wide variety of abnormal conditions—we have been constantly on the alert for a convincing demonstration of their existence in the living animal. Thus far, we have had no success whatsoever, nor has Hanzon (14).

Perhaps our failure to visualize the spaces in the mouse (with which we have had most experience among the mammals) is without significance, since Elias (25) states that they have never been observed in this species. However, the failure of ourselves and others to detect them in the frog—by the technic of fluorescence microscopy—is countered by a seemingly high degree of success on the part of Knisely, Bloch and Warner (32), by direct observation of the normal liver when transilluminated by visible light. They report their observations as follows (p. 39): "The perisinusoidal spaces are frequently visible at 400 to 600× but are very narrow, even when fluid is passing rapidly out through the sinusoid walls. . . . When fluid is not passing into the perisinusoidal spaces the sinusoid walls lie against the hepatic cell cords obliterating the perisinusoidal spaces just as the spaces between the leaves of a book are obliterated, becoming potential spaces when the book is closed." Quite recently, Knisely has reaffirmed his repeated observation, *in vivo*, of the Disse spaces: "One can

see each of them open and close" (27, p. 168). Although Knisely *et al.* (32) studied the liver, *in vivo*, in a number of mice, rats and monkeys, they make no mention of having observed the perisinusoidal spaces in these forms; and Patek (44) was similarly unsuccessful in mice.

On *a priori* grounds, fluorescence microscopy had seemed to us to afford an almost ideal technic for investigating, in the living animal, the sequelae—particularly with respect to the status of the bile canalicular system—of extrahepatic biliary obstruction. Under our conditions, the findings have been remarkably consistent, and would indicate that in obstruction—irrespective of its duration—there is no generalized or, with few exceptions, even localized dilatation of the bile canaliculi. The results with vasoflavine, which is readily transferred in high concentration to the bile canaliculi of the obstructed liver, might be interpreted as supporting the idea of a continuing intrahepatic circulation of the bile. However, vasoflavine is an admittedly unusual compound, and under similar conditions the liver will more or less completely fail to transfer fluorescein, which it normally excretes in large amounts into the bile. The ultimate significance of the vasoflavine pictures we do not know, but at any rate they afford seemingly complete and unequivocal delineation of the bile canaliculi, with no suggestion of canalicular rupture or leakage of the dye into the blood or lymph. That this is the true state of affairs is at least one possibility. However, in these studies we have found ourselves subjected to rather severe limitations. In part, these are imposed by the technic itself; in employing the fluorescence microscope at the relatively high magnification required, the observer is confined to the most superficial portions of the hepatic parenchyma. For the rest, they are imposed by the structural organization of the mouse liver, by which one is permitted access to only limited regions of the hepatic lobules. The definitive account of what happens in the liver in extrahepatic biliary obstruction has yet to be written; and our hopes of contributing significantly thereto have been but partially realized.

SUMMARY

Utilizing the technic of fluorescence microscopy, detailed observations have been made in white mice upon the caliber and character of the bile canaliculi—as delineated by the fluorescent dye, vasoflavine—at varying intervals following ligation and division of the extrahepatic bile ducts, with and without simultaneous removal of the gall bladder. The studies have been accomplished both *in vivo* (under chloral hydrate anesthesia) and immediately after sacrifice of unanesthetized animals.

In mice, under our conditions, the findings have been remarkably consistent; and they would indicate that in extrahepatic biliary obstruction—irrespective of its duration (up to approximately 2 months)—there is no generalized or, with few exceptions, even localized dilatation of the bile canaliculi. Further-

more, we have been unable to visualize any suggestion of canalicular rupture or of leakage of the dye (and so presumably of bile) into the blood or lymph.

Emphasis is placed upon the rather severe limitations encountered in the present study. With the fluorescence microscope, at relatively high magnification, the observer is confined to the most superficial portions of the hepatic parenchyma. Also, the structural organization of the mouse liver is such that, at the surface of the organ, one is permitted access to only limited regions of the hepatic lobules. Under the circumstances, the possible applicability of our findings to other mammals and man, as well as their validity for the mouse itself, must await further investigation.

A description is given of the more important features of the gross and microscopic pathology of extrahepatic biliary obstruction in mice; and the available literature upon biliary obstruction in amphibia and mammals, as studied *in vivo* by fluorescence microscopy, is critically reviewed.

LITERATURE CITED

1. ELLINGER, P. AND HIRT, A.: Mikroskopische Untersuchungen an lebenden Organen. I. Mitteilung. Methodik: Intravitalmikroskopie. Zeitschr. f. Anat. u. Entw., 1929, **90:** 791–802.
2. ELLINGER, P. AND HIRT, A.: Eine Methode zur Beobachtung lebender Organe mit stärksten Vergrösserungen im Luminiszenzlicht (Intravitalmikroskopie). Abderhalden's Handb. d. biol. Arbeitsmeth., 1930, Abt. V, Teil **2/2:** 1753–1764.
3. HARTOCH, W.: Zur Morphologie der Leber- und Nierensekretion. Lebendbeobachtungen im Luminescenzlicht. Zeitschr. f. d. ges. exper. Med., 1931, **79:** 538–547.
4. HARTOCH, W. AND ISRAELSKI, M.: Zur Wirkung der Röntgenstrahlen auf die Funktion sezernierender Organe. Lebendbeobachtung im Luminescenzlicht. I. Mitteilung: Die Leber. Klin. Wchnschr., 1932, **11:** 1464–1465.
5. HARTOCH, W. AND ISRAELSKI, M.: Zur Wirkung der Röntgenstrahlen auf die Funktion sezernierender Organe. Lebendbeobachtungen im Luminiszenzlicht. 1. Mitteilung. Die Leber. Strahlentherapie, 1932, **44:** 557–574.
6. FRANKE, K. AND SYLLA, A.: Mikroskopische Lebendbeobachtungen innerer Organe. I. Mitteilung. Gallencapillar- und Leberzellenstudien mit Mikrophotogrammen am lebenden Frosch. Zeitschr. f. d. ges. exper. Med., 1933, **89:** 141–158.
7. FRANKE, K. AND SYLLA, A.: Mikroskopische Lebendbeobachtung innerer Organe. II. Mitteilung. Beobachtungen an der Leber gesunder und kranker Warmblüter. Zeitschr. f. d. ges. exper. Med., 1934, **93:** 582–599.
8. FRANKE, K.: Klinische und lebendmikroskopische Untersuchungen der gestörten Leberfunktion. I. Gallenfarbstoffuntersuchungen bei Lebererkrankungen mit Ikterus. Zeitschr. f. klin. Med., 1936, **130:** 193–221.
9. FRANKE, K.: Die Technik photographischer Aufnahmen bei mikroskopischen und fluoreszenz-mikroskopischen Lebendbeobachtungen. Abderhalden's Handb. d. biol. Arbeitsmeth., 1938, Abt. V, Teil **10:** 973–986.
10. ANSORGE, I.: Die Ausscheidung von Fluorescein und Trypaflavin durch die Leber des Winterfrosches. Inaug. Diss., Heidelberg, 1933.
11. MARKSTAHLER, H.: Die Ausscheidung von Fluoreszëin und Trypaflavin in der Leber der weissen Ratte. Inaug. Diss., Heidelberg, 1933.

12. HIRT, A.: Über mikroskopische Untersuchungen an der lebenden Leber bei Frosch und weisser Ratte. Anat. Anz., 1934, **78** (Ergänzungsheft): 222–224.
13. HIRT, A., ANSORGE, J. AND MARKSTAHLER, H.: Luminescenzmikroskopische Untersuchungen an der lebenden Frosch- und Rattenleber. Die Ausscheidung von Fluorescein und Trypaflavin. Zeitschr. f. Anat. u. Entw., 1938, **109:** 1–32.
14. HANZON, V.: Liver cell secretion under normal and pathologic conditions studied by fluorescence microscopy on living rats. Acta Physiol. Scand., 1952, 28, Suppl. **101:** 1–268.
15. GRAFFLIN, A. L.: The excretion of fluorescein by the liver under normal and abnormal conditions, observed in vivo with the fluorescence microscope. Am. J. Anat., 1947, **81:** 63–116.
16. GRAFFLIN, A. L.: Studies of fluorescein excretion by the liver under normal and abnormal conditions. *Liver Injury. Transactions of the Seventh Conference.* Hoffbauer, F. W., ed. Josiah Macy, Jr. Foundation, New York, 1948. 73–76.
17. GRAFFLIN, A. L. AND BAGLEY, E. H.: Studies of hepatic structure and function by fluorescence microscopy. Bull. Johns Hopkins Hosp., 1952, **90:** 395–437.
18. GRAFFLIN, A. L.: In vivo studies of hepatic structure and function in the salamander. Anat. Rec., 1953, **115:** 53–62.
19. BAGLEY, E. H. AND GRAFFLIN, A. L.: The mechanism of biliary excretion in mammals. *Liver Injury. Transactions of the Eleventh Conference.* Hoffbauer, F. W., ed. Josiah Macy, Jr. Foundation, 1953. 200–215.
20. MOSES, J. B., EMERY, A. J. AND SCHLEGEL, J. U.: A purified fraction of thioflavine-S (vasoflavine): valuable agent for visualization of blood vessels. Proc. Soc. Exp. Biol. & Med., 1951, **77:** 233–239.
21. SCHLEGEL, J. U.: A new method for demonstration of blood vessels and lymphatics. Anat. Rec., 1949, **103:** 502–503.
22. SCHLEGEL, J. U.: Demonstration of blood vessels and lymphatics with a fluorescent dye in ultraviolet light. Anat. Rec., 1949, **105:** 433–443.
23. MALLOY, H. T. AND EVELYN, K. A.: The determination of bilirubin with the photoelectric colorimeter. J. Biol. Chem., 1937, **119:** 481–490.
24. ELIAS, H.: A re-examination of the structure of the mammalian liver. I. Parenchymal architecture. Am. J. Anat., 1949, **84:** 311–333.
25. ELIAS, H.: A re-examination of the structure of the mammalian liver. II. The hepatic lobule and its relation to the vascular and biliary systems. Am. J. Anat., 1949, **85:** 379–456.
26. ELIAS, H.: The liver cord concept after one hundred years. Science, 1949, **110:** 470–472.
27. ELIAS, H.: Morphology of the liver. *Liver Injury. Transactions of the Eleventh Conference.* Hoffbauer, F. W., ed. Josiah Macy, Jr. Foundation, 1953. 111–199.
28. PFUHL, W.: Die Leber. von Möllendorff's Handb. d. mikr. Anat. d. Menschen, 1932, **5** (Part 2): 235–425.
29. ELIAS, H. AND SOKOL, A.: Dependence of the lobular architecture of the liver on the porto-hepatic blood pressure gradient. Anat. Rec., 1953, **115:** 71–85.
30. LOEFFLER, L. AND NORDMANN, M.: Leberstudien. I. Teil. Die Leber bei der Verdauung von Normalkost, nach Fett-, Glykogen- und Eiweissfütterung, im Hungerzustande und unter der Einwirkung von Adrenalin, Chloroform, Phosphor, Phlorhizin und Insulin. Nach mikroskopischen Untersuchungen der Leber lebender Säugetiere. Virchows Arch., 1925, **257:** 119–181.
31. WAKIM, K. G. AND MANN, F. C.: The intrahepatic circulation of blood. Anat. Rec., 1942, **82:** 233–253.
32. KNISELY, M. H., BLOCH, E. H. AND WARNER, L.: Selective Phagocytosis. I. Microscopic

observations concerning the regulation of the blood flow through the liver and other organs and the mechanism and rate of phagocytic removal of particles from the blood. Kongel. Danske Vidensk. Selsk., Biol. Skrifter, 1948, **4** (Nr. 7): 1–93.

33. RAVDIN, I. S. AND VARS, H. M.: Further studies on factors influencing liver injury and liver repair. Trans. Am. Surg. Assn., 1950, **68:** 42–53.
34. CAMERON, G. R. AND OAKLEY, C. L.: Ligation of the common bile duct. J. Path. and Bact., 1932, **35:** 769–798.
35. ANDREW, W., BROWN, H. M. AND JOHNSON, J. B.: Senile changes in the liver of mouse and man, with special reference to the similarity of the nuclear alterations. Am. J. Anat., 1943, **72:** 199–221.
36. EDLUND, Y. AND HANZON, V.: Demonstration of the close relationship between bile capillaries and sinusoid walls. Acta Anat., 1953, **17:** 105–111.
37. RICH, A. R.: The pathogenesis of the forms of jaundice. Bull. Johns Hopkins Hosp., 1930, **47:** 338–377.
38. EPPINGER, H.: *Die Leberkrankheiten.* Julius Springer, Wien, 1937.
39. LICHTMAN, S. S.: *Diseases of the Liver, Gall Bladder and Bile Ducts.* Lea & Febiger, Philadelphia, 1942.
40. EDLUND, Y.: Studies on the carbohydrate metabolism and liver protection therapy in experimental extrahepatic biliary obstruction. Acta chir. Scand., 1948, **96,** Suppl. 136: 1–187.
41. HIMSWORTH, H. P.: *Lectures on the Liver and its Diseases.* Comprising the Lowell Lectures delivered at Boston, Massachusetts, in March 1947. 2nd Edition. Harvard Univ. Press, Cambridge, 1950.
42. MACGILLAVRY, T. H.: Zur Anatomie der Leber. Sitzungsber. d. kaiserl. Akad. d. Wissensch., Wien, Math.-naturw. Klasse, 1865, **50** (Abth. 2): 207–224.
43. DISSE, J.: Ueber die Lymphbahnen der Säugethierleber. Arch. f. mikr. Anat., 1890, **36:** 203–224.
44. PATEK, P. R.: Circulation of lymph within the liver lobule. Anat. Rec., 1945, **91:** 294.
45. GRAFFLIN, A. L. AND CORDDRY, E. G.: Further studies of hepatic structure and function. Bull. Johns Hopkins Hosp., 1953: In press.

PLATE I

FIG. 1. A composite drawing to illustrate different stages in the injection of the central veins and their tributary sinusoids, as seen at and just below the surface of the liver in the normal mouse. As the injection mass advances into the sinusoids from the central veins, the latter—which rarely achieve an immediately subcapsular position—are obscured from view. For further description see text.

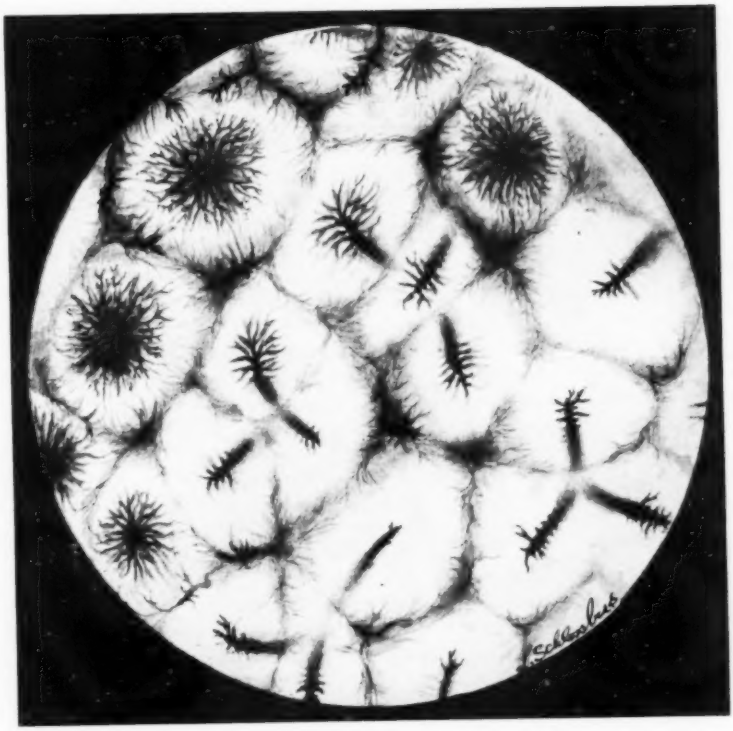


PLATE II

FIG. 2. Mouse A11; common duct ligated and divided, gall bladder removed; sacrificed 11 days later; weight—initial 24.5, final 17.0 gm.

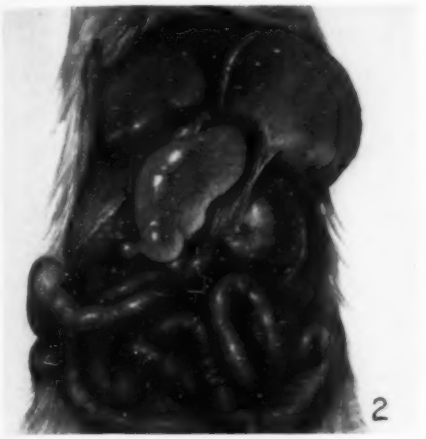
FIG. 3. Mouse A96; common duct ligated and divided, gall bladder removed; sacrificed 20 days later; weight—initial 33.3, final 27.7 gm. Excerpt from protocol: Liver is normal in size, color and consistency. The duodenum and a portion of the pancreas are loosely adherent to a smooth, glistening, thin-walled, multilobular, transparent cystic mass situated adjacent to the porta of the liver, and measuring 10 mm. in length, 6 mm. in width and 4 mm. in antero-posterior diameter. All 3 ties are easily identified and found to be secure.

FIG. 4. Mouse A84; common duct ligated and divided, gall bladder removed; sacrificed 67 days later; weight—initial 20.2, final 21.3 gm. Excerpt from protocol: Liver appears normal in size, color and consistency, with a smooth surface. It is displaced laterally and posteriorly by a large, thin-walled, transparent, glistening, multiloculated mass, measuring 27 mm. in length, 8 mm. in width, and 9 mm. in antero-posterior diameter. Coursing across the mid-portion of the sac is the densely adherent duodenum, with no evidence of fistulous communication or of obstruction. The dilated cystic duct arises from the supero-ventral surface of the main mass, and measures 6 mm. in diameter at its base, 2 mm. at its apex, and 10 mm. in length. Transillumination of the liver reveals cystic dilatations throughout the substance of the upper lobes. Aspiration of the cystic mass shows it to be divided into 4 compartments, with no demonstrable intercommunication. Compartment contents: 1—0.8 ml., slightly turbid, yellow-brown; 2—0.5 ml., faintly turbid, green tinge; 3 (cystic duct)—0.29 ml., same as in 2; 4—0.26 ml., almost water-clear, very faint green tinge.

FIG. 5. Mouse A89; common duct ligated and divided, gall bladder removed; sacrificed 67 days later; weight—initial 31.0, final 27.2 gm. Excerpt from protocol: Liver appears grossly normal. A large, thin-walled, glistening, transparent, multilobular cystic mass fills the entire epigastrium, and measures c. 20 mm. in length, 17 mm. in width, and 20 mm. in antero-posterior diameter. The cystic duct is visible on the supero-ventral aspect of the mass, and is similar in appearance. It measures 7 mm. in length and 3 mm. in width at its widest point; the tie is secure on its distal end. The remaining 2 ties are identifiable on the ventro-lateral aspect of the sac, 5 mm. apart; on the ventro-medial aspect is a 4 x 5 mm. hemorrhagic area within its wall; there is no evidence of rupture, leakage or fistula formation.

FIG. 6. Mouse A98; common duct ligated and divided, gall bladder left *in situ;* sacrificed 31 days later; weight—initial 31.3, final 29.0 gm. Excerpt from protocol: Left upper and lower lobes of liver appear normal; there is a small area of necrosis in the right upper lobe, and a large one (5 x 10 mm.) in the right middle lobe; the right lower lobe is entirely replaced by a large necrotic mass. The entire upper abdomen is filled with a large, glistening, thin-walled, transparent, greenish mass measuring 20 mm. in length, 14 mm. in width, and 16 mm. in antero-posterior diameter. Above this mass is the large, dilated gall bladder, measuring 16 mm. in length and 6 mm. in diameter at the fundus. The cystic duct is 2 mm. in diameter at its widest point. Aspiration of the sac, which is monolocular and in communication with the gall bladder, yields 1.7 ml. of cloudy, light green fluid. All ties can be identified and are secure, and there is no evidence of fistula formation.

FIG. 7. Mouse A72; common duct ligated and divided, gall bladder left *in situ;* sacrificed 66 days later; weight—initial 27.0, final 25.1 gm. Excerpt from protocol: The liver appears grossly normal. The upper abdomen is filled with a large, smooth, glistening, very thin-walled cystic mass, measuring 22 x 18 mm., and in free communication via the dilated cystic duct with the gall bladder, which is c. 4× normal size. Aspiration of the cystic mass and gall bladder yields 1.4 ml. of slightly turbid, pale green fluid. The 2 ligatures can be identified and are secure, and there is no evidence of fistula formation.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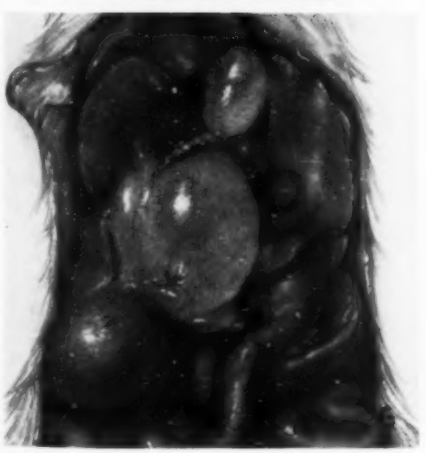

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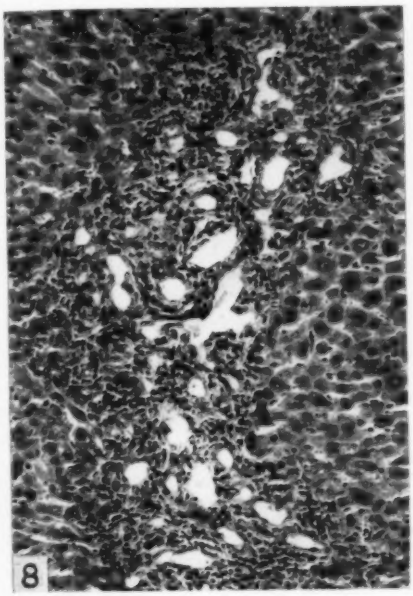
PLATE III

FIG. 8. Mouse A75; common duct ligated and divided, gall bladder removed; sacrificed 27 days later; weight—initial 28.8, final 25.4 gm. Fairly typical area of periportal infiltration, showing proliferation and apparent dilatation of small bile ducts. Formalin fixation, paraffin section, 5 μ, hematoxylin and eosin. × 150. Excerpt from protocol: The liver appears normal in size, color and consistency. There is a dilatation of the extrahepatic ducts measuring 13 mm. in length and 5 mm. in diameter. Aspiration of the sac yields a slightly turbid fluid with fine mucus threads and pale yellowish-green in color. All ties can be identified and are secure, and there is no evidence of fistula formation.

FIG. 9. Mouse A68; common duct ligated and divided, gall bladder removed; sacrificed 36 days later; weight—initial 27.3, final 25.0 gm. Periportal area showing extensive cellular infiltration and numerous small channels lined with squamous epithelium, and presenting an almost angiomatous appearance (see text). Formalin fixation, paraffin section, 5 μ, hematoxylin and eosin. × 150. Excerpt from protocol: The liver appears small and has a smooth surface. A tremendous thin-walled sac fills the entire upper abdomen as well as the right and left flanks. The sac contains greenish fluid and grey mucus shreds 3–4 mm. in length. Upon opening the sac, all ties can be identified and are secure; there is no evidence of fistula formation.

FIG. 10. Mouse A72; common duct ligated and divided, gall bladder left *in situ;* sacrificed 66 days later; weight—initial 27.0, final 25.1 gm. Irregular valve-like formations in wall of tremendously dilated intrahepatic bile duct. Formalin fixation, paraffin section, 5 μ, hematoxylin and eosin. × 100. For excerpt from protocol, see legend of Fig. 7.

FIG. 11. Mouse A15; common duct ligated and divided, gall bladder removed; sacrificed 15 days later; weight—initial 29.3, final 24.7 gm. A striking group of valve-like formations in a markedly dilated intrahepatic bile duct. Formalin fixation, paraffin section, 5 μ, hematoxylin and eosin. × 150. Excerpt from protocol: There is a large dilatation of the extrahepatic ducts measuring c. 14 mm. in diameter. The sac contents are faintly tinged with bile. All ties can be identified and are secure.



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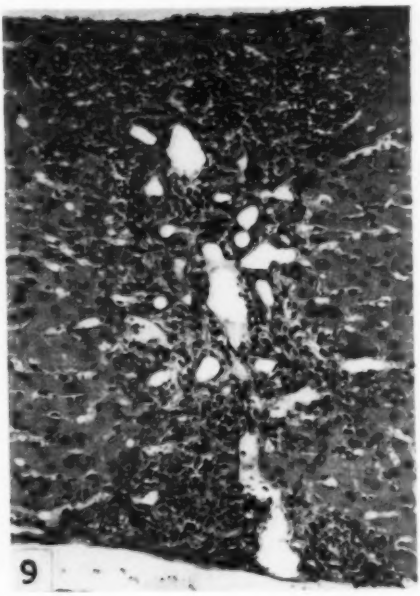

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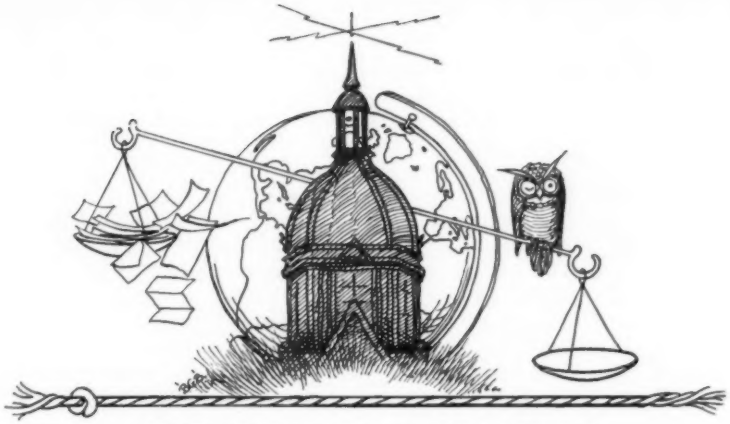

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

The Collected Papers of Adolf Meyer. Four Volume Set $30.00. Edited by EUNICE WINTERS. Volume 4, Mental Hygiene. With an introduction by Alexander H. Leighton. 585 pp. The Johns Hopkins Press, Baltimore, Md.

Mental hygiene was one of the major fields of Adolf Meyer's interest and work. He was closely concerned personally in efforts to guide constructively the movement so energetically sparked by Clifford Beers, and he contributed much to the formulation of professional and scientific principles which gave depth and significance to the movement. His writings on this topic, in a book of nearly 600 pages, form the fourth and final volume of his Collected Papers.

The following section headings, under which the editor, Miss Winters, has arranged these papers, exhibits the wide range covered, and their heterogeneous character: Ideals for Work in Mental Hospitals, Psychiatry and the Law, Psychiatry and the Community, Aftercare and Social Work, The Mental Hygiene Movement, Eugenics, Psychiatry and the Child, and Psychiatry and the Problems of Maturity.

The difficulties of arrangement reflect the difficulties of development of mental hygiene concepts and practices, for that development has not proceeded in a straightforward and logical fashion from clearly established and generally accepted scientific findings, but from the flashes of insight and the disciplined sense of certain leaders, prominent among them Dr. Meyer himself. Perhaps the mental hygiene movement was pushed into existence a bit hastily and prematurely, and has suffered somewhat for its opportunistic origins.

For the understanding of the development of Dr. Meyer's thinking about mental hygiene, two threads of logic could be traced through these papers, one dealing with the "consensus," to which the subject index supplies a series of references, and the other dealing with the discrepancy between ambition and achievement, for which the index supplies little guidance.

Dr. Meyer had great respect for the onsensus. He stressed repeatedly that good judgment and good health are favored by the habit of respectful consideration of the opinions and attitudes of well-informed persons. The earnest search for such a consensus, as between physician and patient, forms the essential feature of his brilliant and widely-known con-

tribution to the therapy of paranoid conditions (1913, Collected Papers, Vol. II, pp. 517–560), and his preoccupation with the further development of this principle was reflected in his writings during the years when he provided intellectual guidance for the mental hygiene movement.

The line of thought concerned with "constructive composure," as a satisfying realistic balancing of interests and ambitions with actual performance, is well presented in the brief address (pp. 241–248) to the 1923 National Conference of Social Work.

The introduction to this volume, by Alexander Leighton, provides for the reader a thoughtful and penetrating analysis of the Meyerian contributions to mental hygiene, made graciously personal by touches of tender adulation.

JOHN C. WHITEHORN

Pitkin's Conduction Anesthesia, 2nd edition. Edited by JAMES L. SOUTHWORTH, ROBERT A. HINGSON AND WINIFRED M. PITKIN. 1005 pp., $22.50. J. B. Lippincott Co., Philadelphia, Pa.

This very comprehensive work covers almost every phase of conduction anesthesia. It is a useful text for the student or the specialist, either in surgery or anesthesiology. In fact it is about the only such comprehensive and up to date text of its kind available today. It covers very thoroughly the anatomy involved, the pharmacology of the local anesthetics, and the techniques for producing local anesthesia in every portion of the body for just about any surgical procedure.

It is profusely illustrated with what in general are extremely satisfactory diagrams and photographs.

In some instances the book proves rather awkward to use. Sometimes it is necessary to search in several different sections of the book to gain all of the information required for the performance of a particular anesthetic procedure. That is to say, it may be necessary to search out details of the anatomy in the section devoted to this subject and then details on the technique in a later section of the book. The index is very helpful in this regard; but not always so. For instance although 27 pages of the book, an entire section, are devoted to techniques for brachial-plexus block, if one searches for brachial-plexus block in the index there is no such heading. The profusion of techniques offered for different anesthetic procedures is sometimes more confusing than helpful. Not always is it clearly indicated which procedure is preferable, or under which circumstances one procedure may be more useful than another.

There are 14 pages of bibliography at the end of the book for the use of the student who wishes to read the sources from which the author has drawn his material, or the original work involved in the development of a technique.

On the whole, this is an extremely useful textbook, but one may hope that in a subsequent edition it may be somewhat simplified from the point of view of ready accessibility of material to the physician anxious to make use of a specific anesthetic technique.

DONALD F. PROCTOR

Topographische Ausdeutung Der Bronchien Im Röntgenbild. By CLAUS ESSER. 152 pp., Kartoniert DM 33. Georg Thieme Verlag, Stuttgart, Germany.

The main body of this book is devoted to a detailed and exhaustive description of the anatomy of each of the major bronchial divisions in each lung. These excellent descriptions are combined with a very large number of extremely good illustrations and diagrams. There are many bronchographic demonstrations of the bronchial tree which are some of the best that have ever been published. The accompanying diagrams help to orient the reader as to the direction from which the pictures are taken.

At the end of the book there is a brief but very good bibliography of articles by other authors on this subject.

All things considered, for the reader who is anxious to obtain a better orientation as to the complicated problem of the tracheo-bronchial anatomy, this is the best illustrated text which has come to the attention of this reviewer to date.

DONALD F. PROCTOR

A Method of Anatomy, 5th edition. By J. C. BOILEAU GRANT. 870 pp., $7.00. The Williams & Wilkins Co., Baltimore, Md.

The fifth edition of Grant's *Method of Anatomy* continues Dr. Grant's pleasantly informal exposition of anatomy. Seventy-eight new illustrations have been added. Through revision and deletion only 17 additional pages appear, and the book remains approximately the same size as in the past. He retains an introductory general section of two chapters offering a systemic glimpse with a number of physiological highlights. This is followed by six sections covering the six regional divisions traditionally associated with the dissection of the human body. The book ends with a section on miscellaneous items concerning the autonomic nervous system, body types, bones of the skull, bones of the pectoral girdle, and bones of the carpus. This has been the general plan throughout the earlier editions. The line drawings which characterize the illustrations are simple, clear, and instructive.

This book is not a textbook of descriptive anatomy in the encyclopaedic sense, and, as Dr. Grant states in the preface to the first edition, "it involves certain departures from tradition." It is regional rather than systemic in its approach, and discusses structures as they are met in a regional dissection, as a teacher of Dr. Grant's skill would discuss these matters in the laboratory. It intentionally omits many of the details encountered in the traditional anatomical textbook, and thereby acquires a brevity and clarity impossible in those books. Most students will find it a source of assistance in their anatomical studies, and will appreciate the correlation of facts and ideas it contains.

The new edition is thoroughly up-to-date, and remains a valuable supplementary text within the scope of its intent. The reviewer does not find in it a text to supplant the traditional textbook of descriptive anatomy, however. There will always be a necessity for a systemic review of structures encountered, and all of the details of the traditional descriptive textbook will from time to time be required for an understanding of many relations and variations encountered in a dissection.

It should be noted that no attention is given to the central nervous system in this book, beyond discussions of the spinal and cranial meninges. The hypophysis cerebri is listed in the index as appearing on page 619, but is not mentioned on that page; and along with the pineal body, carotid body, and glomus coccygeum appears to have been omitted.

ELBERT B. RUTH

Surgery of the Oblique Muscles of the Eye. By WALTER H. FINK. 350 pp., $8.75. The C. V. Mosby Co., St. Louis, Missouri.

Analysis of a case of squint, and corrective surgery therefore, is based in large part on quantitative data concerning (1) the average anatomical relationships of the extraocular muscles, and (2) deviations from the average. In Part I of his monograph, Dr. Fink presents a detailed but easily readable summary of previous literature on the anatomy of the inferior and superior oblique muscles. He supplements this review with considerably more exact statistical data from careful measurements he has made of 100 anatomical specimens. These original data, which represent years of work, should survive as one of the lasting fundamental bases for future analyses of ocular movements.

Part II, which deals with the clinical analysis and surgery of oblique muscle defects,

reflects in large part Dr. Fink's current opinions, based on his broad experience in this, as yet, largely controversial field.

JACK S. GUYTON

Handbook of Tropical Dermatology and Medical Mycology. Vol. I. Edited by R. D. G. PH. SIMONS. 845 pp., $15.00. Elsevier Press, New York.

This is the first volume of the first edition of a book which had its origin in a prisoner of war camp in the Pacific Theatre of World War II. The book is the work of a group of 40 international authorities in dermatology, venereology and tropical diseases, each author taking a chapter covering some phase of tropical dermatology. The editor introduces the subject with a long first chapter entitled "General Survey" which is comprehensive and well illustrated. There are chapters on the pediatric aspects, on pigmentary disturbances, syphilis, bejel, yaws, pinta, goundou, leprosy, leg ulcers, leishmaniasis, tuberculosis, plague, rickettsial diseases, miliaria and tropical acne. The chapters on leprosy, leishmaniasis and yaws are all inclusive and interesting. Some of the other chapters are short and do not seem to cover the subject matter completely. Since this is a first edition, the editor will doubtless improve these chapters in subsequent editions.

The book is written concisely and clearly and has many beautiful illustrations. It is a valuable reference book for the internist and dermatologist. The next volume will include the chapters on fungus diseases omitted from this first volume.

EUGENE S. BERESTON

Rypins' Medical Licensure Examinations. Topical Summaries and Questions, 7th edition. Edited by WALTER L. BIERRING. 856 pp., $8.00. J. B. Lippincott Co., Philadelphia, Pa.

In his prefatory remarks Dr. Rypins points out that the medical school is often in a much better position to assess the student's academic and encyclopaedic knowledge than are the state and national medical boards and that the board examinations be reserved to test the graduate's ability to apply that knowledge to the practice of clinical medicine. Dr. Bierring adds that the purpose of the examination, besides the application of knowledge to clinical problems, is to determine the general fitness of the individual to practice the art and science of medicine. One might easily ask how often these objectives are attained in the examinations as they are now held, varying as they do from state to state. In most of them it is the academic and encyclopaedic knowledge that is primarily tested. In this book the authors have presented what they believe to be a representative and useful series of questions to guide and stimulate the thought of the prospective candidate.

The work is divided into twelve sections, the first seven of which deal with the basic medical sciences and the latter five with the clinical sciences. Each selection of questions is preceded by a summary of the subject, incorporated in about 50 to 60 pages of reasonably large print; so it can be seen that a subject is often treated in an extremely brief fashion. Repetition has been avoided as much as possible although this often necessitates consulting several sections to answer one question. The tantalizing and annoying brevity is unfortunately inevitable in a book of this type. To answer a question of the treatment of pernicious anemia it is necessary to refer to the section on pharmacology, the section on medicine, the section on biochemistry, and the section on pathology. Then many would disagree with the therapeutic oral dosage of Vitamin B_{12} given as 1 microgram a day.

The sections are edited by well-known and competent authorities and are, on the whole concise, accurate and well arranged. However, in summaries such as precede the questions, there is little room for such verbal extravagances as "teetering on a bed-pan" and "the acrobatics of balancing on a bed-pan" within 4 pages of each other. What to include and

what to omit must have been a great problem to the authors. One could list many omissions especially in the therapeutic side of the section of medicine; for example the use of oxygen in coronary occlusion, procaine amide in arrythmias, ACTH in asthma, hexamethonium and apresoline in hypertension. On the other hand most of these subjects are touched on in the section of pharmacology. The author of the section on medicine must have had one of the most difficult tasks keeping to his allotted space. To pharmacology is allotted 79 pages and medicine only 62 pages. In the section on biochemistry the metabolism of potassium is merely touched on.

In summary, there is no doubt that the questions are representative and useful for a prospective candidate to work through, but the summaries will have limited appeal and one might wonder if specimen answers might not have been more successful.

JOHN B. MACGIBBON

Anzeige und Ausführung der Eingriffe an Ohr, Nase und Hals. Edited by HELLMUT UFFENORDE. With a chapter on surgical treatment of impaired hearing by Horst Wullstein. 540 pp., Ganzl. DM 79.80. Georg Thieme Verlag, Stuttgart, Germany.

The first edition of this book appeared in 1942, written by the father of the editor of the present, second, edition. Extensive revisions of text and illustrations have been made, to record the rapid increase in knowledge; and a chapter has been added on fenestration and other tympano-plastic surgery. The illustrations, especially of x-ray films and photomicrographs of lesions, add much to the value of the text for the student and the young specialist in this field. For the young otolaryngologist who reads German the book should prove very helpful, even though some of the recommended procedures are not the ones favored in some institutions.

GEORGE TH. NAGER

BOOKS RECEIVED FOR REVIEW

A Collection of Urogenital Drawings. By WILLIAM P. DIDUSCH. 222 pp. American Cystoscope Makers, Inc., New York.

Die Geburtshilflichen Operationen. By HEINRICH MARTIUS. 290 pp., Ganzleinen DM 34. Georg Thieme Verlag, Stuttgart, Germany.

Die Lungenresektionen. By A. LEZIUS. 143 pp., Ganzleinen DM 49.80. Georg Thieme Verlag, Stuttgart, Germany.

Die Neuzeitlichen Brustwand—und Extremitäten—Ableitungen in der Praxis. By HERBERT REINDELL AND HELMUTH KLEPZIG. 188 pp., Ganzleinen DM 24.60. Georg Thieme Verlag, Stuttgart, Germany.

Differentialdiagnose innerer Krankheiten. By ROBERT HEGGLIN. 556 pp., Ganzleinen DM 55. Georg Thieme Verlag, Stuttgart, Germany.

The Intimate Life. By J. NORVAL GELDEHUYS. With a Preface by Dr. CHARLES R. ERDMAN. 96 pp., $2.75. Philosophical Library, New York.

Medicine, Vols. I and II. Edited by Hugh G. Garland and William Phillips. Foreword by F. A. E. CREW. 2146 pp., $25.00. St. Martin's Press, New York.

Organic Chemistry, Vols. III and IV. Edited by HENRY GILMAN. 1245 pp., $8.75 each. John Wiley & Sons, New York.

The Physician in Atomic Defense. Atomic Principles, Biologic Reaction and Organization for Medical Defense. By THAD P. SEARS. With a Foreword by JAMES J. WARING. 308 pp., $6.00. Year Book Publishers, Chicago, Illinois.

Mr. Tompkins Learns the Facts of Life. By G. GAMOW. 88 pp., $2.75. Cambridge University Press, New York.